

Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations

Graduate Studies

5-1983

Studies in Peptide Synthesis Towards the Lanthionine Analog of Des-N-Tetramethyl Triostin A

Ganesh Devdas Kini
Utah State University

Follow this and additional works at: <https://digitalcommons.usu.edu/etd>

 Part of the [Chemistry Commons](#)

Recommended Citation

Kini, Ganesh Devdas, "Studies in Peptide Synthesis Towards the Lanthionine Analog of Des-N-Tetramethyl Triostin A" (1983). *All Graduate Theses and Dissertations*. 7249.
<https://digitalcommons.usu.edu/etd/7249>

This Dissertation is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



STUDIES IN PEPTIDE SYNTHESIS TOWARDS THE LANTHIONINE
ANALOG OF DES-N-TETRAMETHYL TRIOSTIN A

by

Ganesh Devdas Kini

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Chemistry

UTAH STATE UNIVERSITY
Logan, Utah

1983

DEDICATION

With reverence to my mother, Sushila Devdas Kini, and my father, Devdas Vasudev Kini, this thesis is dedicated. My first teachers, they provided the impetus and support for my entire education, without which this work would be impossible.

ACKNOWLEDGEMENTS

The author wishes to express his heartfelt gratitude and appreciation

- to Dr. Richard K. Olsen, the research director, for his advice, help and support
- to Drs. Michael P. Windham, Daniel L. Comins, William M. Moore and Thomas M. Farley, committee members
- to Dr. Krishna L. Bhat, for his fine "lab company," technical advice and discussions
- to Dr. William J. Hennen, for his valuable suggestions and help in "getting started"
- to Jeffrey L. Johnson, for his technical help
- to the National Institute of Health, for financial support of the project
- to Mr. Dayanand A. Prabhu and Mr. Manjunath S. Nayak, for their encouragement, help and moral support
- and last but not least, to Susan L. Herron for typing the dissertation competently at short notice.

Ganesh Devdas Kini

TABLE OF CONTENTS

	Page
DEDICATION.	ii
ACKNOWLEDGEMENTS.	iii
LIST OF SCHEMES	vii
ABSTRACT.	viii
INTRODUCTION.	1
LITERATURE REVIEW	8
The structure and conformation of echinomycin and triostin A	8
Synthesis of triostin and echinomycin.	10
Synthesis of lanthionines.	17
Synthesis of unsymmetrical cystine containing peptides.	22
RESULTS AND DISCUSSION.	27
Preparation of lanthionine <u>27</u> from N,N'-di-t-butyloxycarbonyl-L-cystine dimethyl ester	29
The mechanism of lanthionine contraction	31
Synthesis of and cyclization studies of linear octapeptides <u>29</u> and <u>30</u>	32
Studies on lanthionine contraction of tetrapeptide <u>34</u> , hexapeptide <u>35</u> , cyclic octapeptide <u>13</u> and TANDEM.	35
The unsymmetrical approach to the synthesis of <u>4</u>	37
Contraction of unsymmetrical disulfides.	38
Synthesis of lanthionine by nucleophilic displacement of -chloro-L-alanine	41
Synthesis of lanthionine by displacement of O-activated serine derivatives with cysteine	47
Synthesis of unsymmetrical lanthionines <u>76</u> and <u>80</u> using thiol sulfinates	54
CONCLUSION.	68
EXPERIMENTAL.	70

	Page
Synthesis of N,N'-di-tert-butyloxy-carbonyl-L-cystine dimethyl ester (<u>26</u>)	71
Synthesis of N,N-di-tert-butyloxy-carbonyl-L-lanthionine dimethyl ester (<u>27</u>)	71
Synthesis of hexapeptide lanthionine <u>28</u>	72
Synthesis of octapeptide lanthionine <u>29</u>	74
Attempted synthesis of octadepsipeptide <u>31</u> from <u>29</u>	75
Synthesis of octapeptide <u>32</u>	76
Attempted synthesis of cyclic octapeptide <u>31</u> from <u>32</u>	77
Synthesis of tetrapeptide <u>34</u>	78
Lanthionine contraction of tetrapeptide <u>34</u>	79
Synthesis of hexapeptide <u>35</u>	79
Attempted lanthionine contraction of above hexapeptide <u>35</u>	80
Synthesis of N-benzyloxycarbonyl-L-seryl-L-valine methyl ester.	81
Synthesis of N-benzyloxycarbonyl-O-(p-toluene sulfonyl)-L-seryl-L-valine methyl ester (<u>61</u>).	81
Attempted displacement of <u>61</u> with benzyl mercaptan	82
Displacement of N-benzyloxycarbonyl-O-(p-toluenesulfonyl)-L-seryl-glycine methyl ester with benzyl mercaptan	83
Synthesis of tripeptide <u>63</u>	83
Synthesis of tripeptide mesylate <u>65</u>	84
Attempted displacement of <u>65</u> with benzyl mercaptan	85
Synthesis of N-benzyloxycarbonyl-L-serine-(N-t-butyloxycarbonyl)-hydrazide (<u>66</u>)	85
Synthesis of N-Benzyloxycarbonyl-O-(p-toluenesulfonyl)-L-serine (N-t-butyloxycarbonyl) hydrazide (<u>67</u>)	86
Attempted displacement of <u>67</u> with benzyl mercaptan	87
Synthesis of N,N'-di-t-butyloxy-carbonyl-L-cystine di-t-butyl ester (<u>72</u>)	87
Synthesis of N-t-butyloxycarbonyl-L-cysteine t-butyl ester (<u>70</u>).	88
N,N'-Di-benzyloxycarbonyl-L-cystine diethyl ester (<u>73</u>)	89
Synthesis of thiol sulfinate <u>74</u>	89
Synthesis of N-benzyloxycarbonyl, N'-t-butyloxycarbonyl-L-cystine ethyl, t-butyl diester (<u>75</u>).	90
Synthesis of lanthionine <u>76</u>	90
N,N'-di-2,2,2-trichloroethyloxy-carbonyl-L-cystine di-t-butyl ester (<u>77</u>)	91

	Page
Synthesis of thiol sulfinic acid <u>78</u>	91
Synthesis of N-benzyloxycarbonyl-L-cysteine ethyl ester	92
Synthesis of N-benzyloxycarbonyl, N'-2,2,2-trichloro-ethyloxycarbonyl-L-cystine ethyl t-butyl diester (<u>79</u>)	93
Synthesis of lanthionine <u>80</u>	93
Synthesis of N-2,2,2-trichloro-ethyloxycarbonyl-L-cysteine t-butyl ester (<u>86</u>)	94
Alternate method of synthesis of <u>79</u>	94
REFERENCES.	95
APPENDIX.	103
VITA.	105

LIST OF SCHEMES

Scheme	Page
1	11
2	30

ABSTRACT

Studies in Peptide Synthesis Towards the Lanthionine

Analog of Des-N-Tetramethyl Triostin A

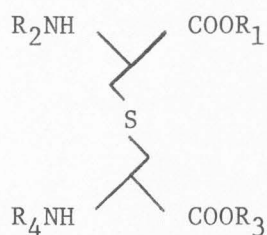
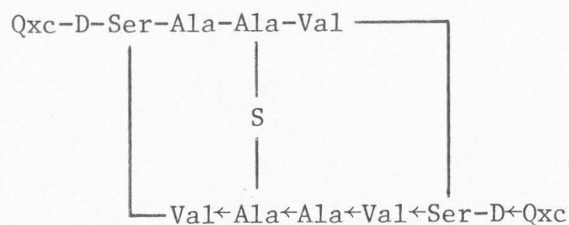
by

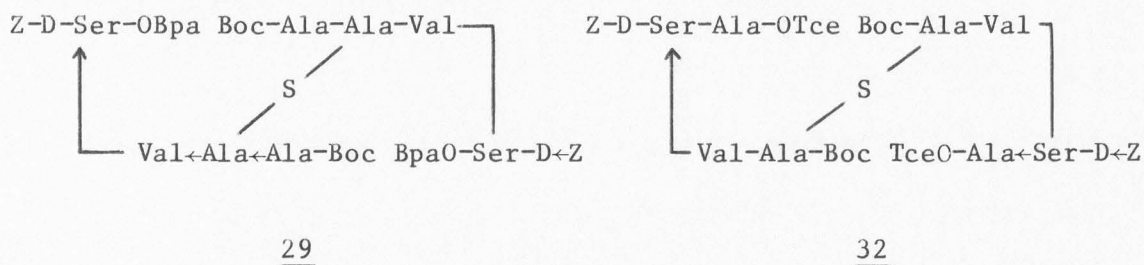
Ganesh Devdas Kini, Doctor of Philosophy

Utah State University, 1983

Major Professor: Richard K. Olsen
 Department: Chemistry

Studies have been carried out towards the synthesis of bicyclic octadidepsipeptide lanthionine 4, an analog of des-N-tetramethyl triostin A. Starting with a symmetrical lanthionine unit 5 ($R_1, R_3 = \text{Me}$; $R_2, R_4 = \text{t-butyloxycarbonyl}$), synthesized from the corresponding cystine by contraction with hexaethylphosphorus triamide, linear octadidepsipeptides 29 and 32 were synthesized.

5, 76, 804



Attempted cyclizations of 29 and 32 to the bicyclic octapeptide were unsuccessful.

The failure of the symmetrical approach to 4 prompted the synthesis of an unsymmetrical lanthionine unit with appropriate compatible protecting groups. Attempts to synthesize unsymmetrical lanthionines by nucleophilic displacement on N-benzyloxycarbonyl- β -chloro-L-alanine and appropriate O-activated serine derivatives with the thiol function of L-cysteine were unsuccessful. L-valine as the C-terminal amino acid of the O-activated serine was found to sterically hinder the nucleophilic displacement with the thiol, since the displacement could be effected when glycine was present in lieu of valine.

The reaction of thiolsulfinate with cysteine led to an unsymmetrical cystine which was then contracted with hexaethylphosphorus triamide to yield the corresponding lanthionines. The synthesis of unsymmetrical lanthionines 76 (R_1 = t-butyl, R_2 = t-butyloxycarbonyl, R_3 = ethyl, R_4 = benzyloxycarbonyl) and 80 (R_1 = t-butyl, R_2 = 2,2,2-trichloroethyloxycarbonyl, R_3 = ethyl, R_4 = benzyloxycarbonyl) has been accomplished. Lanthionines 76 and 80 each have four different protectings on the amino and carboxyl functions. These can be selectively removed in the right sequence for a proposed

synthesis of 4. Further studies towards the synthesis of 4, as proposed, were not pursued.

(115 pages)

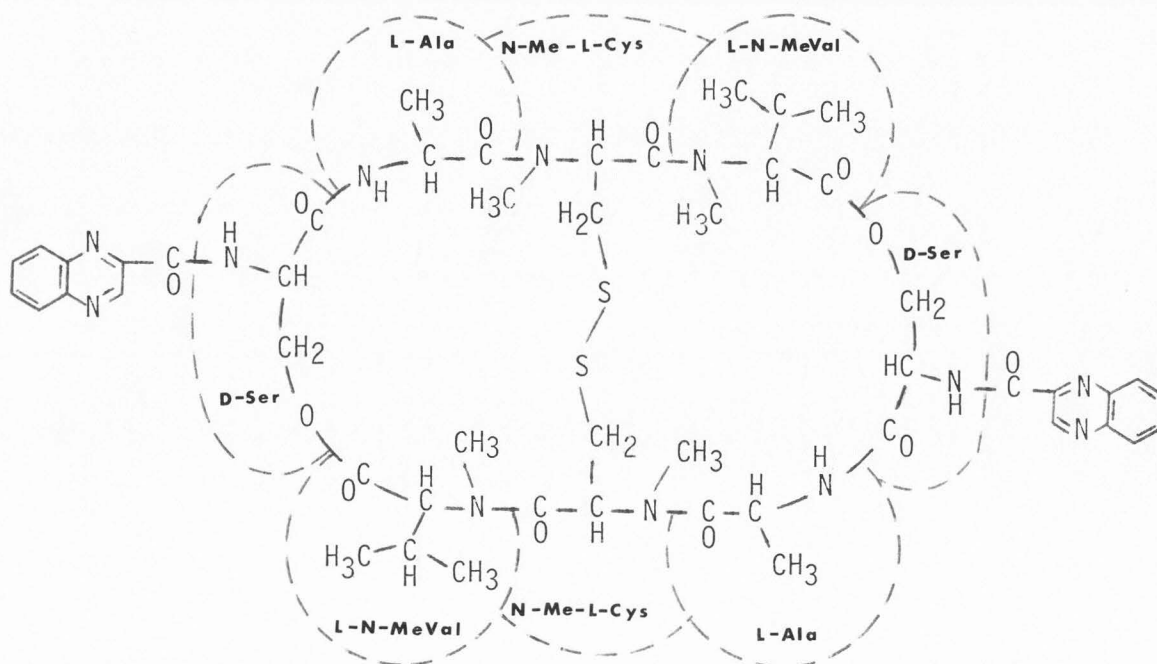
INTRODUCTION

The quinoxaline antibiotics are a class of compounds, characteristic of which is the presence of one or more quinoxaline chromophores. They are produced by several species of streptomycetes. Isolated and reported independently by Carter et al.¹ and Ueda et al.² in 1954, these are a group of peptide antibiotics that possess unusual bicyclic structures. In 1961, the existence of two families of quinoxaline antibiotics, the quinomycins and the triostins, was reported.^{3,4}

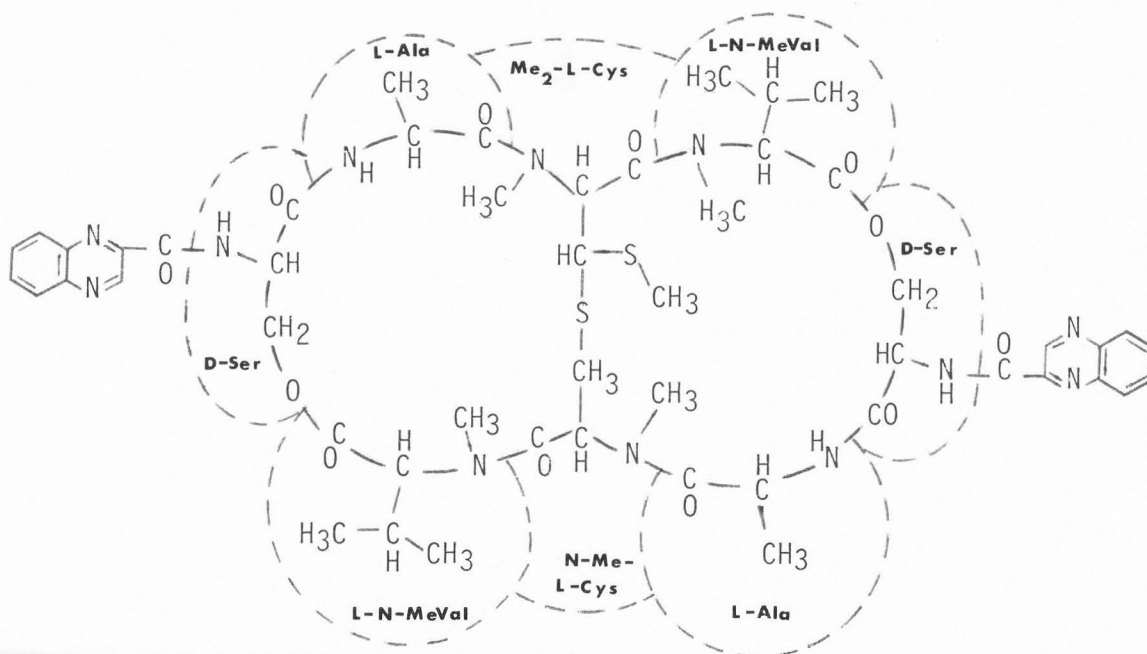
The structures of triostin A (1) and echinomycin (2) are as shown. Both are octadidepsipeptides, each composed of two D-serine, two L-alanine, two N-methyl-L-valine and two N-methyl-L-cysteine units. The carboxyls of the N-methyl-L-valine units form two ester bonds, called depsipeptide bonds in peptide terminology, with the respective hydroxyls of the D-serine units. To the amine nitrogen of each D-serine unit is attached a quinoxaline-2-carbonyl unit.

The two families of antibiotics differ, however, in the central bridging of the bicyclic structure. Triostin A has a disulfide linkage between the two N-methyl-L-cysteine units. Hence, it is a symmetrical molecule with a C_2 axis of rotation passing through the disulfide bond. Echinomycin, on the other hand, has a unique and hitherto unknown dithioacetal linkage in lieu of the disulfide bridge in triostin A. Consequently, it lacks the element of symmetry present in the triostin A molecule.

Echinomycin, also known as quinomycin A, is produced by several species of streptomycetes, while triostin A is produced only by



Triostin A

1

Echinomycin (quinomycin A)

2

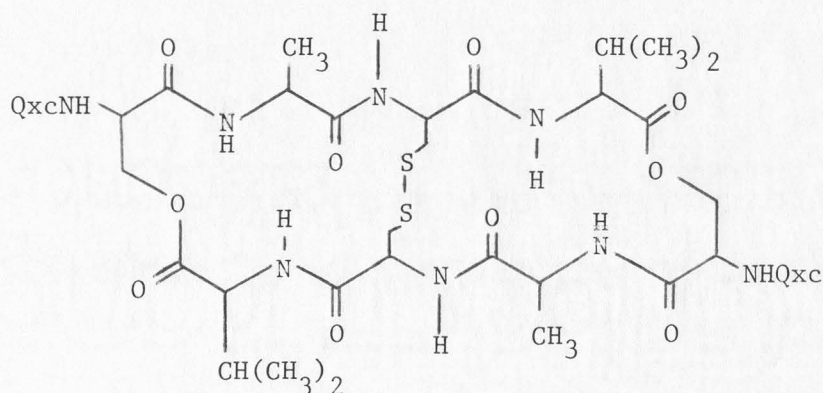
Streptomyces aureus. The quinoxaline antibiotics are of considerable interest because of their potent biological activity, which has been reviewed by Katagiri et al.⁵ They are powerful antibiotics exhibiting antiviral, antimicrobial and cytotoxic activity. Their biological significance was noticed in 1955, when antitumor activity was first demonstrated.⁶ A mixture of triostin A and triostin C has been shown to be particularly effective against the mouse tumor strain, Mecca lymphoma. While the quinoxalines are known to show activity against a fairly broad spectrum of gram-positive, acid fast and anaerobic bacteria, their activity towards gram-negative bacteria and fungi is insignificant.^{3,4}

The antibiotics have also been found to be active towards mycoplasma,⁷ polio virus infection in mice,⁸ and protozoa.^{2,9} That they inhibit multiplication of bacteriophages has also been reported.¹⁰ Studies were carried out by Katagiri and Sugivra¹¹ and Matsuura,¹² to determine the antitumor activity exhibited by some of the quinoxalines against several tumor systems in mice, rats, hamsters and chickens. These studies indicated quinoxaline C to be the most therapeutically active. Another important aspect found from these studies was the fact that minor variations in structure within the quinoxalines resulted in significantly different antitumour activity. Echinomycin is now in the first phase of preclinical evaluation by the National Cancer Institute.¹³ Olsen¹⁴ has recently compiled a review of the quinoxaline group of antibiotics.

The biological activity of the quinoxalines has aroused considerable interest in their possible clinical uses as also in their mode of biological action. First clues to their possible mode of

action were provided by their cytotoxic response. Poisoned cell strains, upon staining, showed the aggregation of chromatin and dispersion of the nuclei.¹⁵ This effect was common to all of the quinoxaline antibiotics. Waring et al.^{16,17,18,19} proposed the mechanism of activity to be direct interaction with DNA (deoxyribonucleic acid). The hypothesis was that the two quinoxaline-2-carbonyl units are positioned parallel to each other and in a plane perpendicular to the peptide backbone. When they point in the same direction, the molecule resembles a staple. The staple-shaped molecule binds by inserting both the quinoxaline residues between different sets of base pairs on the DNA molecule, thereby inhibiting RNA synthesis. This unusual form of binding, termed "bifunctional intercalation" has been thoroughly studied and supported by binding data,^{16,17} X-ray crystallographic data¹⁹ and solution conformation studies.²⁰

The biological properties and the unusual mode of activity of the quinoxaline antibiotics together have generated considerable interest in the total chemical synthesis of these compounds as well as analogs. The first total synthesis of a quinoxaline antibiotic, triostin A was accomplished by Olsen and Chakravarty.¹⁵ Olsen and coworkers²¹ also synthesised the first biologically active analog, des-N-tetramethyltrioestin A. This synthetic analog, with the acronym TANDEM, differs from the naturally occurring antibiotic triostin A by the absence of the four N-methyl groups on the two cysteine and two valine residues (structure 3).

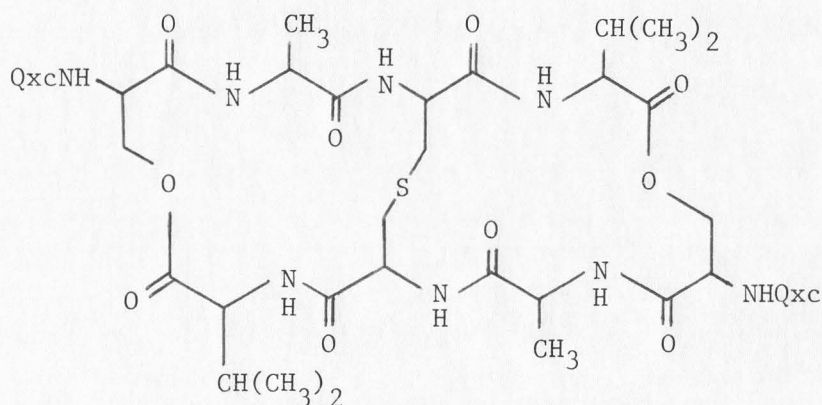


TANDEM [Des-N-Tetramethyl Triostin A]

3

The binding properties of TANDEM have turned out to be very unique. TANDEM exhibits a high degree of binding specificity for DNA with alternating sequences of adenine and thymine (poly dA-dT), as against the low specificity for poly (dG-dC).^{16,17} Interestingly, the other naturally occurring quinoxaline antibiotics show the opposite sequence specificity in their binding to DNA. Waring²² has recently reviewed the sequence selective binding to DNA of echinomycin, triostin A and related quinoxaline antibiotics in relation to their structure.

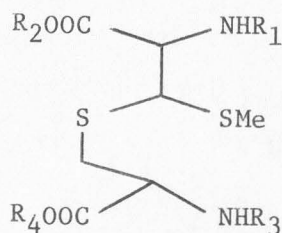
The research presented in this thesis deals with studies towards the synthesis of another analog of the quinoxaline antibiotics, the lanthionine analog of des-N-tetramethyl trisotin A (structure 4).



Lanthionine Analogue of TANDEM

4

The interest in the synthesis of this molecule is two fold. One, the approach is to start with a lanthionine unit and develop a peptide synthesis, which could then be applied to a preformed β -methylthiolanthionine, structure 5. Compound 5 is the central bridging

5

moiety of echinomycin 2. This mixed dithioacetal has, as yet, not been synthesised, and its chemical properties, like stability to reaction conditions, have not been documented. In the previously reported synthesis of TANDEM²¹ the cyclic octapeptide is first synthesized with the formation of the disulfide bridge being the

penultimate step in the total synthesis. The approach here is exactly the opposite, to start with the central sulfur containing unit and develop a synthesis around it.

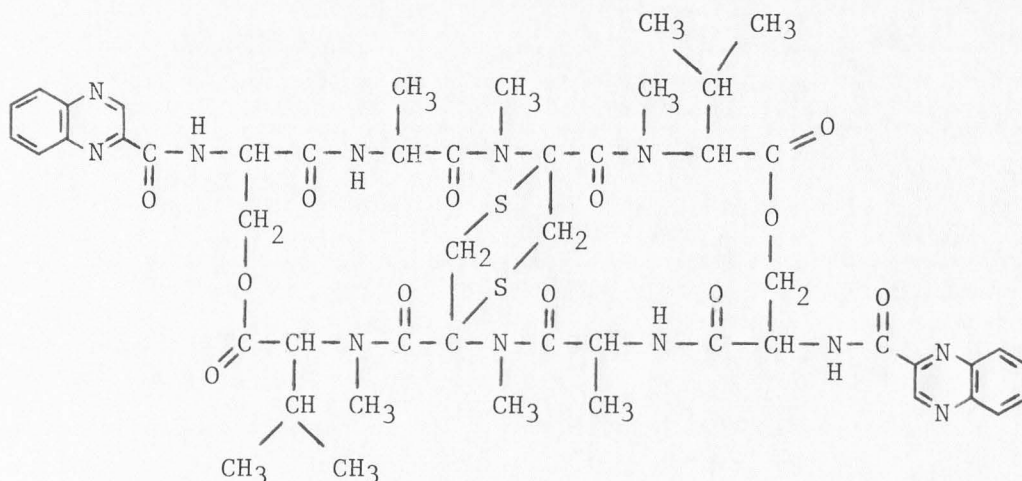
The second aspect of interest in this study is the possible manifestation of the binding properties of the lanthionine analog. TANDEM, which differs from naturally occurring triostin A by the absence of four N-methyl groups, shows opposite sequence specificity in binding to DNA as compared to triostin A. The lanthionine analog would have its central bridge shortened by one sulfur atom compared to triostin A, but would exist with the same number of atoms in the bridging group as in echinomycin. Hence, it would be of considerable interest to study the binding properties of the lanthionine analog in comparison with TANDEM itself, as also with triostin A and echinomycin.

Attempts to synthesize 4 starting with a symmetrical lanthionine unit were unsuccessful. Hence, the logical alternative was to start with a suitably protected unsymmetrical lanthionine. The results of these studies will be presented herewith.

LITERATURE REVIEW

The structure and conformation of echinomycin and triostin A

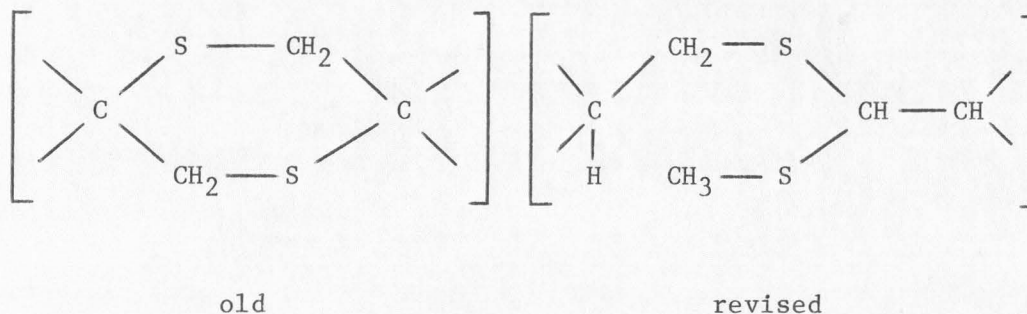
Prelog and coworkers²³ reported the first structure elucidation for a quinoxaline antibiotic. From the data available at that time, they proposed the following structure 6 for echinomycin. The sulphur unit was proposed to exist as a 1,4-dithiane ring system.



6

In 1975, Williams and coworkers,²⁴ and Martin and coworkers²⁵ independently reported their spectroscopic studies on the structure of echinomycin. After consideration of their evidence based on ¹H and ¹³C nuclear magnetic resonance data, the hitherto accepted 1,4-dithiane cross link was revised to a dithioacetal cross link. Structure 2 is now the accepted structure for echinomycin. As seen in 2, there exists a chiral center within the cross link at the β-carbon of the lantionine unit. The configuration at the chiral carbon, however, has not yet

been established. The revision in the structure of echinomycin is schematically shown below, with the rest of the molecule remaining the same.



Otsuka and Shoji²⁶ carried out structural studies on the triostins and assigned structure to triostin C. Among the triostins produced by Streptomyces aureus, triostin C happens to be the major isomer. After further studies on the minor components of the quinoxaline antibiotics, Otsuka et al.²⁷ assigned structure to triostin A in 1967.

There are a number of recent reports on the conformations of triostin, echinomycin and the quinoxaline antibiotics in general.^{28,29,30,31} Ughetto and Waring,³² have reported theoretical studies carried out on the conformations of echinomycin. From these studies, it was concluded that conformational changes are restricted by the dithioacetal linkage, and that the two quinoxaline chromophores orient themselves on the same side of the peptide ring in the conformation that is most stable. Williamson and Williams³¹ have come to the same conclusion for the solution conformation of echinomycin, after analysis of nmr data using the nuclear Overhauser effect, Feeney and coworkers³³ have published proton nmr data supporting Waring and Ughetto's conclusion.

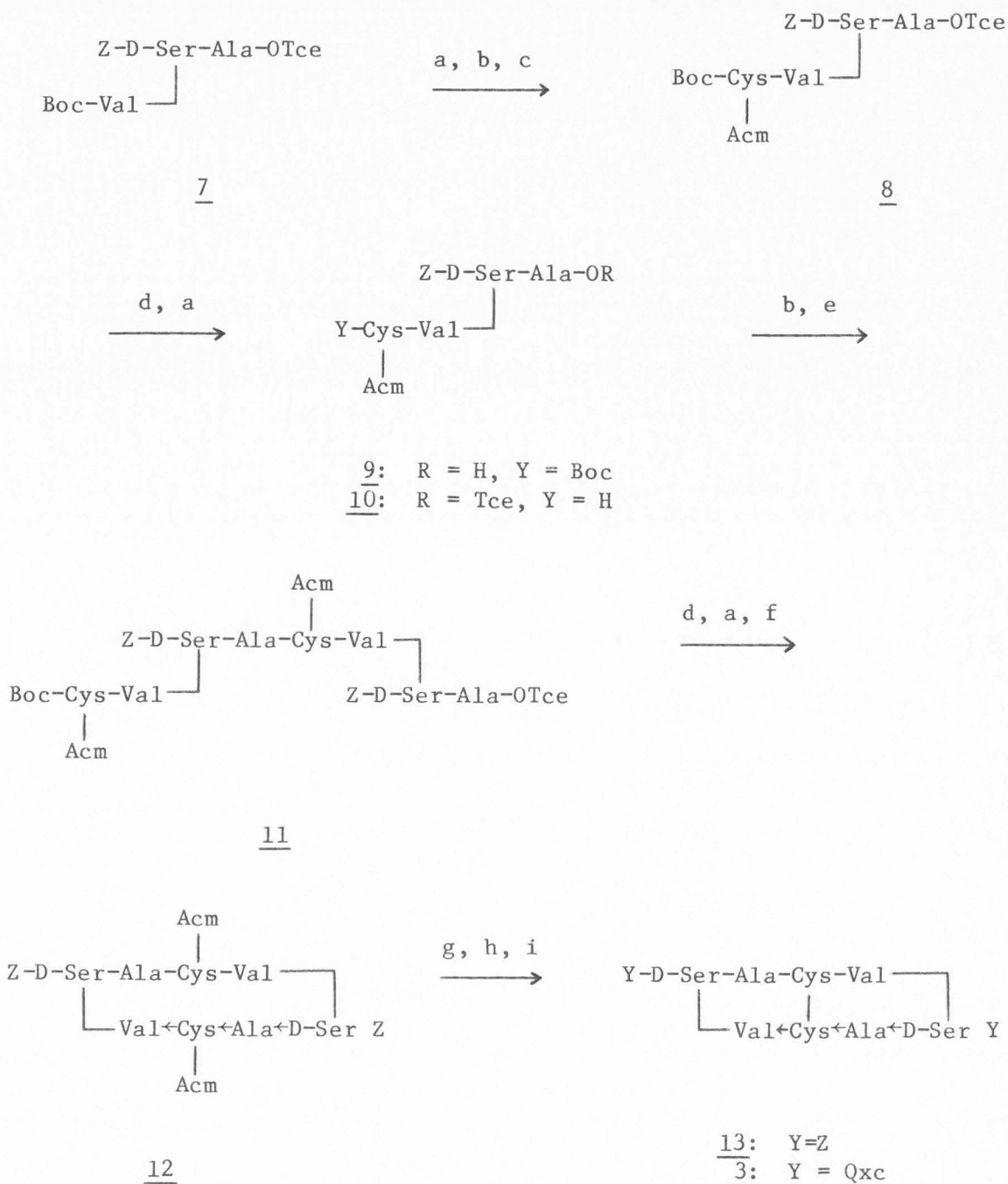
Williams and coworkers,³⁴ and Kawano and coworkers³⁵ have reported studies on the solution conformation of triostin A. They found triostin A to exist in two symmetrical conformations in chloroform solution. From the analysis of their nmr data, Williams and coworkers²⁰ concluded that the quinoxaline chromophores, like in echinomycin, were oriented on the same side of the peptide backbone. Olsen,¹⁴ in his review on the quinoxaline antibiotics, has discussed the conformational analysis for triostin and echinomycin in detail.

Synthesis of triostin and echinomycin

There have been relatively few reports on synthetic studies of the quinoxaline antibiotics. To date, the total synthesis of echinomycin has not been reported.

Olsen and coworkers³⁶ were the first to synthesize a biologically active analog of a naturally occurring quinoxaline antibiotic. This analog of triostin A differed from the naturally occurring antibiotic by the absence of four N-methyl groups on the two valine and two cysteine residues. Hence, it was christened des-N-tetramethyltriosin A, with the acronym TANDEM (structure 3). The synthesis was carried out according to Scheme 1.

The linear tetrapeptide 8, which constitutes one half of the symmetrical molecule TANDEM 3, was a key intermediate. Tetrapeptides 9 and 10, both derived from 8, were then coupled to give the linear octapeptide 11. This is an example of how Olsen and coworkers took advantage of the symmetry in the molecule to facilitate its synthesis. Other salient features of this synthesis were the tactful use of protecting groups. The 2,2,2-trichloroethyl ester³⁷ protecting group at the alanine residue in 11 can be selectively hydrolyzed under



(a) TFA; (b) NaHCO₃; (c) Boc-Cys(Acm)-OH, EDC, HOBT, THF; (d) Zn, aq*ACOH; (e) EDC, HOBT, THF; (f) DCC, N-methylmorpholine 1-hydroxy succinimide, THF; (g) I₂, MeOH; (h) HBr, AcOH; (i) Et₃N, DMF, QxcCl, Abbreviations: Boc = tert-butyloxycarbonyl, Acm = acetamidomethyl, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide*HCl, HOBT = N-hydroxybenzotrizole, THF = tetrahydrofuran, DMF = N,N-dimethylformamide, Qxc = quinoxaline-2-carbonyl, Z = benzyloxycarbonyl.

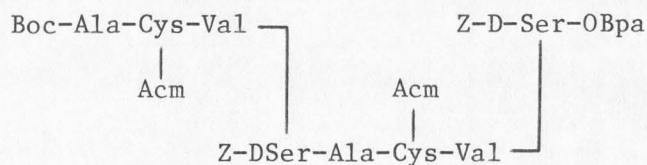
Scheme 1.

reductive conditions with zinc and acetic acid without affecting the depsipeptide ester bond. The acetamidomethyl group^{38,39} was used to protect the cysteine thiol functions. This group undergoes oxidative cleavage upon treatment with iodine in methanol with the simultaneous formation of the disulfide bond⁴⁰ (12 to 13).

The amino groups of the two serines were protected with the benzyloxycarbonyl group (Z group), while the N-terminals of the cysteine residues were protected with the tert-butyloxycarbonyl (Boc) group.⁴¹ The latter can be selectively cleaved with trifluoroacetic acid⁴² without affecting the Z-groups and the depsipeptide ester bonds. The use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in coupling reactions facilitates the workup, as compared to that with the use of N,N'-dicyclohexylcarbodiimide. In the latter case, one of the products of the reaction, N,N'-dicyclohexylurea, is insoluble and precipitates out of the solvents normally used in peptide couplings, like methylene chloride or pyridine. This product has to be removed by repeated filtration. When EDC is used, the urea formed is water soluble, and thus can be removed from the reaction mixture by washing the organic phase with water. The depsipeptide bond in 7 was formed by the use of N,N'-dicyclohexylcarbodiimide in anhyd pyridine.⁴³ Compound 13 was converted to 3 by acidolysis of the benzyloxycarbonyl group using HBr in acetic acid followed by acylation⁴⁴ with 2-quinoxaloyl chloride in dimethylformamide.

The synthesis of the des-N-tetramethyl analog was prompted by the fact that N-methylamino acids are generally not commercially available in protected form as are their naturally occurring counterparts.

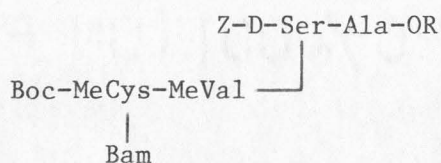
Olsen and coworkers⁴⁵ also reported another approach to the synthesis of des-N-tetramethyltrioestin A. Octapeptide 12 was synthesized, the cyclization being effected by the coupling of the C-terminal of serine with the N-terminal of alanine. The linear octapeptide synthesized had the following structure,



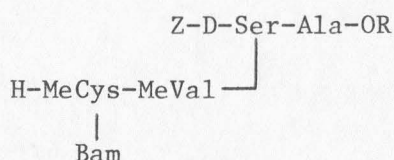
Bpa: p-bromophenacyl

The p-bromophenacyl ester⁴⁶ protecting group (Bpa) was used and found to be more suitable than the trichloroethyl ester group.

Chakravarty and Olsen¹⁵ accomplished the total synthesis of trioestin A, using the strategy and methods developed for the synthesis of TANDEM, with a few, but significant, modifications. A new protecting group, the benzamidomethyl (Bam) group was developed⁴⁷ for the protection of the thiol functions of the N-methyl-L-cysteine residues. This group was found to be much better than the acetamidomethyl used in the synthesis of TANDEM. Tetrapeptide 14, again the key intermediate, upon treatment with zinc and acetic acid to synthesize 15, did not undergo a clean transformation, and starting material 14 was left behind.

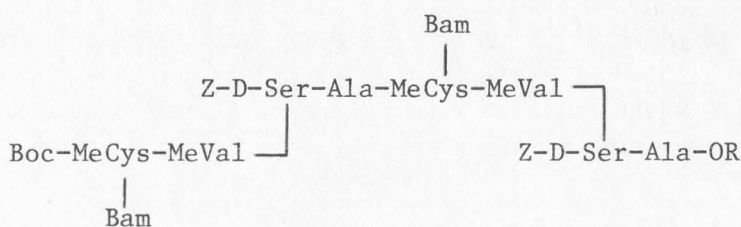


14: R = Tce
15: R = H



16: R = Tce

17: R = H (as trifluoroacetate salt)

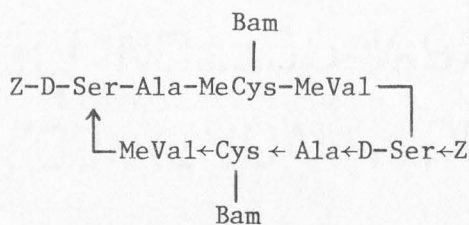


18: R = Tce

19: R = H

The problem of incomplete removal of the trichloroethyl ester function was found to be more predominant in the synthesis of 19 from 18. Conditions more vigorous than normally used for the removal of the trichloroethyl ester function resulted in the destruction of 18 to form a complex mixture of products. Hence, this pathway to the cyclic octapeptide had to be modified.

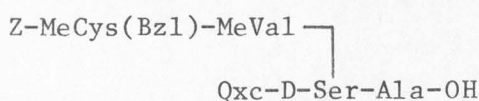
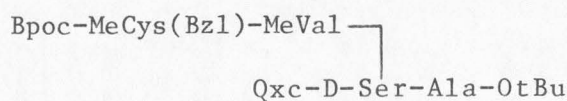
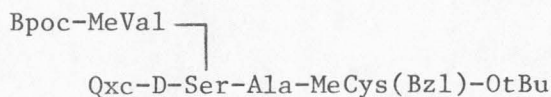
Linear octadepsipeptide 19 was synthesized in good yield by coupling 17 with the mixed anhydride of 15.



20

Cyclic octapeptide 20 was synthesized from 19, after deblocking the amino group of the N-methylcysteine, followed by cyclization in THF solution with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide.⁴⁸ During the course of this synthesis, Chakravarty and Olsen observed that, in general, N-methylamino acid peptides were oils as compared to their naturally occurring counterparts.

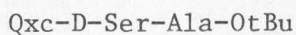
Shin and coworkers⁴⁹, as part of their studies towards the total synthesis of triostin A, synthesized the tetradepsipeptides 21, 22, 23, and 24.

21222324

Bpoc: 2-(4-biphenyl)-2-propoxycarbonyl
Qxc: quinoxaline-2-carbonyl

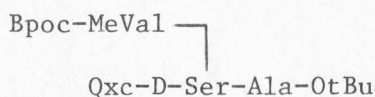
In the design of their synthesis, the symmetry of the triostin A molecule was considered. Cyclization by the formation of the depsipeptide ester bonds was not considered, since the low reactivity of the serine hydroxyl would necessitate the activation of the appropriate carboxyl group⁵⁰ to an extent that side reactions and racemization would be facilitated. Hence, the cyclization had to be effected by the formation of a peptide bond. Each of the peptides 21,

22, 23 and 24 represent one half of the triostin A molecule. The approach was to attempt synthesis of triostin A via 21 and 22 or 23 and 24 in two separate systems. Attempted deprotection of 22 to yield the free amine resulted in the formation of dipeptide 25, and a product identified as cyclo[-MeCys(Bzl)-MeVal-]. These were inferred to be products of intramolecular aminolysis. Since then, Shin and coworkers⁵¹ have reported the total synthesis of triostin A in 1980.



25

The above workers also developed a new method for the introduction of the quinoxaline-2-carbonyl group on the serine nitrogen by the use of the p-nitrophenyl ester of quinoxaline-2-carboxylic acid. An interesting observation was the formation of the depsipeptide bond in peptide 26 by the use of N,N'-carbonyldiimidazole⁵² or 2,2'-dimethyl-



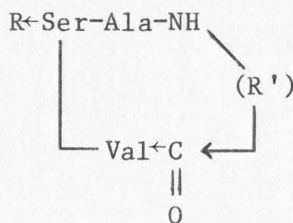
26

1,1'-carbonyldiimidazole⁵³ even though in relatively low yield. This is contrary to the findings of Olsen and coworkers⁴³ who found these methods unsuccessful in the formation of depsipeptide bonds.

Olsen⁵⁴ reported studies on the introduction of the quinoxaline-2-carbonyl moiety in amino acids, tripeptides and tetrapeptides. The Merrifield Solid Phase method⁵⁵ was found to give satisfactory results as compared to standard methods of peptide coupling.

Cheng and coworkers⁴⁴ investigated synthetic routes to bis-quinoxaloyl derivatives containing peptide linkages.

Olsen and coworkers⁴³ synthesized cyclic tetradepsipeptides as models for one portion of the bicyclic octadepsipeptide echinomycin.

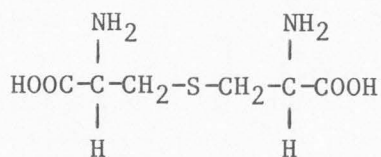


R: carbobenzoxy, quinoxaline-2-carbonyl
 R': $-(\text{CH}_2)_4-$

The depsipeptide bond formation was accomplished by the use of an excess of the carboxylic acid with N,N'-dicyclohexylcarbodiimide in pyridine solution. The mixed anhydride and carbonyldiimidazole methods were found to be unsatisfactory.

Synthesis of lanthionines

The amino acid lanthionine has the following structure. This

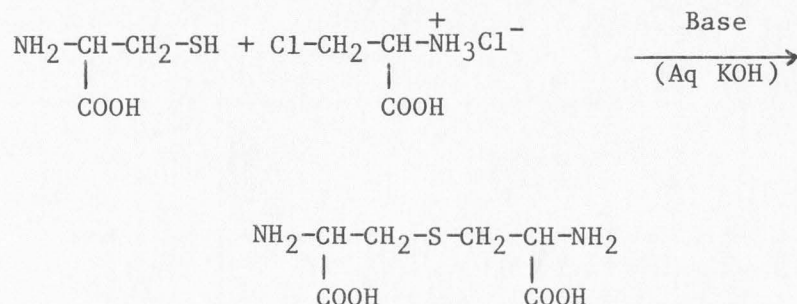


amino acid, β -amino- β -carboxyethyl sulfide, was first reported by Horn and coworkers^{56,57} to be formed by the action of sodium carbonate on wool and other proteins.

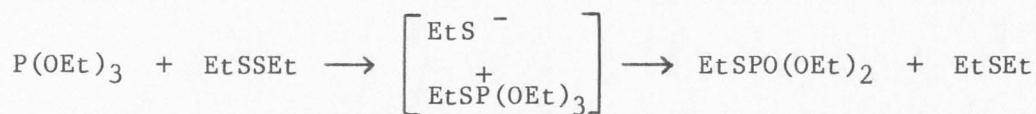
Lanthionine is a special case of a sulfide, hence an unsymmetrical lanthionine would be a special case of an unsymmetrical sulfide. There

have been several methods reported for the synthesis of unsymmetrical sulfides.⁵⁸⁻⁶² However, these are not amenable to peptide synthesis and to the synthesis of lanthionines. The methods reported for the synthesis of symmetrical and unsymmetrical lanthionines and cysteinyl peptides will be discussed.

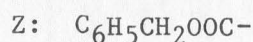
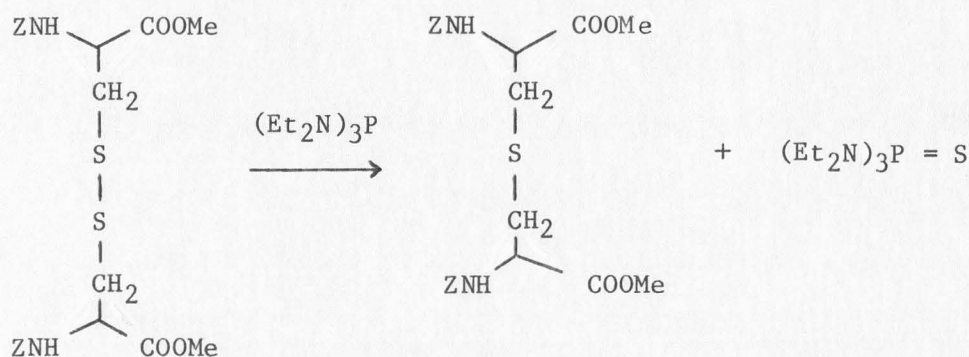
Brown and du Vigneaud⁶³ reported the first synthesis of lanthionine by the action of L-cysteine on dl- α -amino- β -chloropropionic acid in strongly alkaline base.



Jacobsen and coworkers⁶⁴ reported, as early as in 1955, the use of phosphines to contract disulfides.

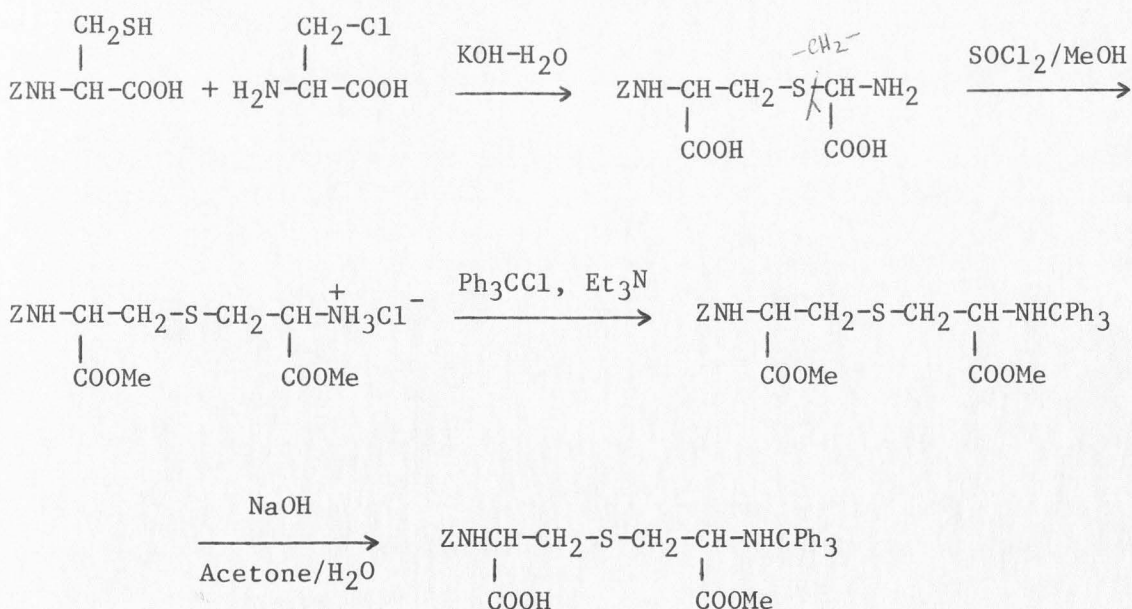


In 1971, Harpp and Gleason⁶⁵ utilized alkylamino phosphines to effect selective removal of a sulfur from appropriate cystine derivatives to yield symmetrical lanthionines.



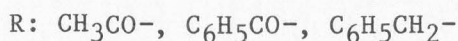
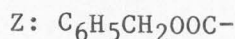
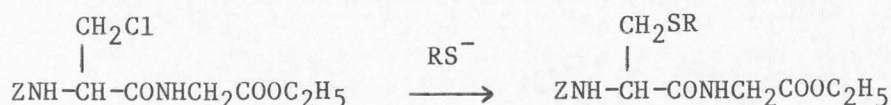
The method of Harpp and Gleason, however, was reported⁶⁵ not to be useful for the synthesis of unsymmetrical lanthionines. This reaction will be discussed in detail later.

Photaki and coworkers⁶⁶ synthesized an unsymmetrical lanthionine by making use of the steric bulk of the triphenylmethyl group (trityl). A lanthionine diester, with a trityl group on one of the α -amino functions, upon hydrolysis, yields a mono-acid, the trityl group shielding the ester on the α -carboxyl from hydrolysis. Photaki's reaction sequence was as follows.



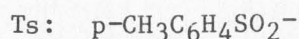
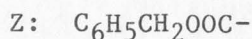
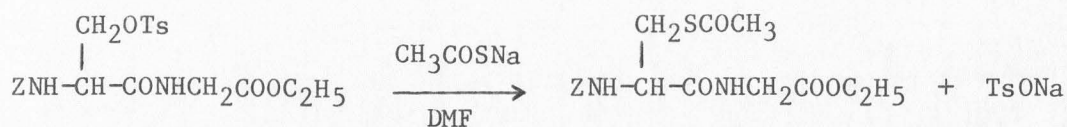
As will be discussed later, attempts to synthesize a lanthionine using this method were unsuccessful.

Wilchek and coworkers⁶⁷ reported the synthesis of optically active L-cysteine peptides in high yields by the reaction of thio reagents with peptides containing a β -chloroalanine unit. The thio reagents used included thioacetate, thiobenzoate and benzyl mercaptide. N,N-dimethylformamide or ethyl acetate were used as solvents.

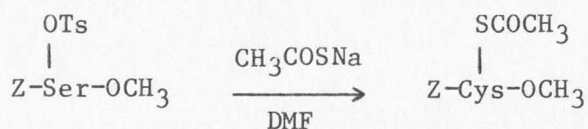


While this method worked well for the synthesis of some cysteinyl peptides, it could not be used on systems with an ester function on the carbon β - to the carbon with the chlorine.

In a strikingly similar manner, Wilchek and coworkers⁶⁸ synthesized cysteinyl peptides from peptides with an L-serine residue. The thioacetate anion effected an $\text{S}_\text{N}2$ displacement on O-p-toluene-sulfonyl-L-serine peptides in N,N-dimethylformamide or aqueous media to quantitatively yield optically active S-acetyl-L-cysteine peptides.

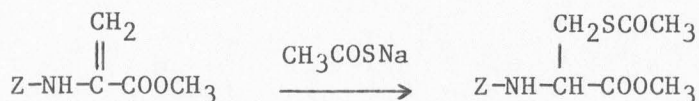
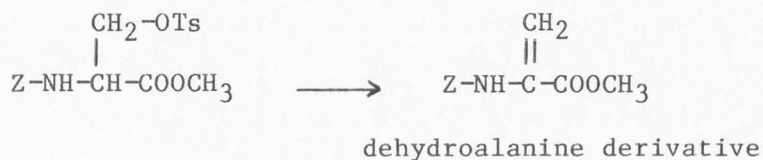


However, an ester function β to the carbon with the tosylate group led to racemic product.



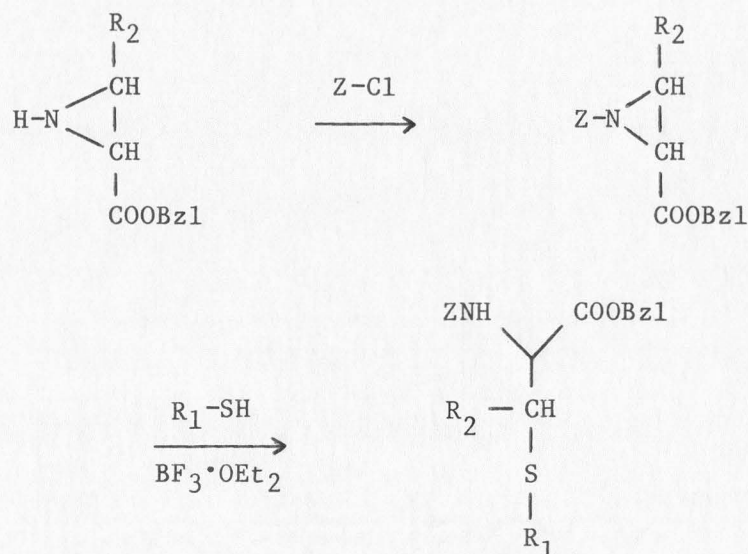
Ser: serine
Cys: cysteine

The ester function promotes elimination to yield a dehydroalanine ester. The mercaptide anion then adds to the α,β -unsaturated ester in Michael fashion to yield racemic product. This is one of the



limitations of this method. Results on the attempted synthesis of some cysteinyl peptides using this method will be presented and discussed later.

Nakajima and coworkers⁶⁹ recently reported the first example of ring opening by thiols to yield lanthionine. The aziridine ring, upon reaction with thiols under Lewis acid catalyzed conditions, yielded the corresponding lanthionines.

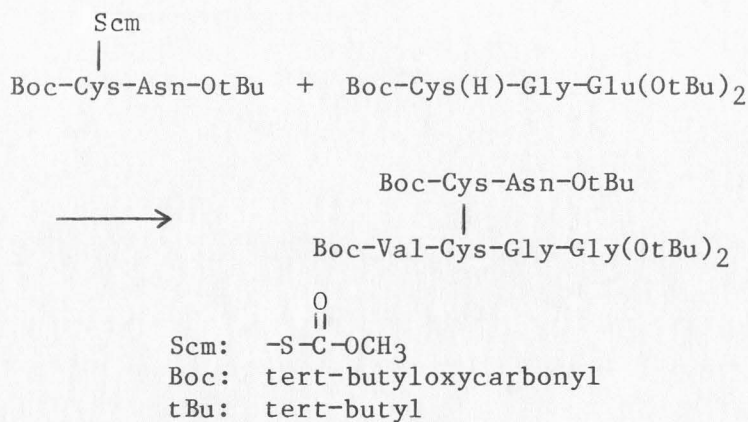


R_1 : Alkyl
 R_2 : H, CH_3

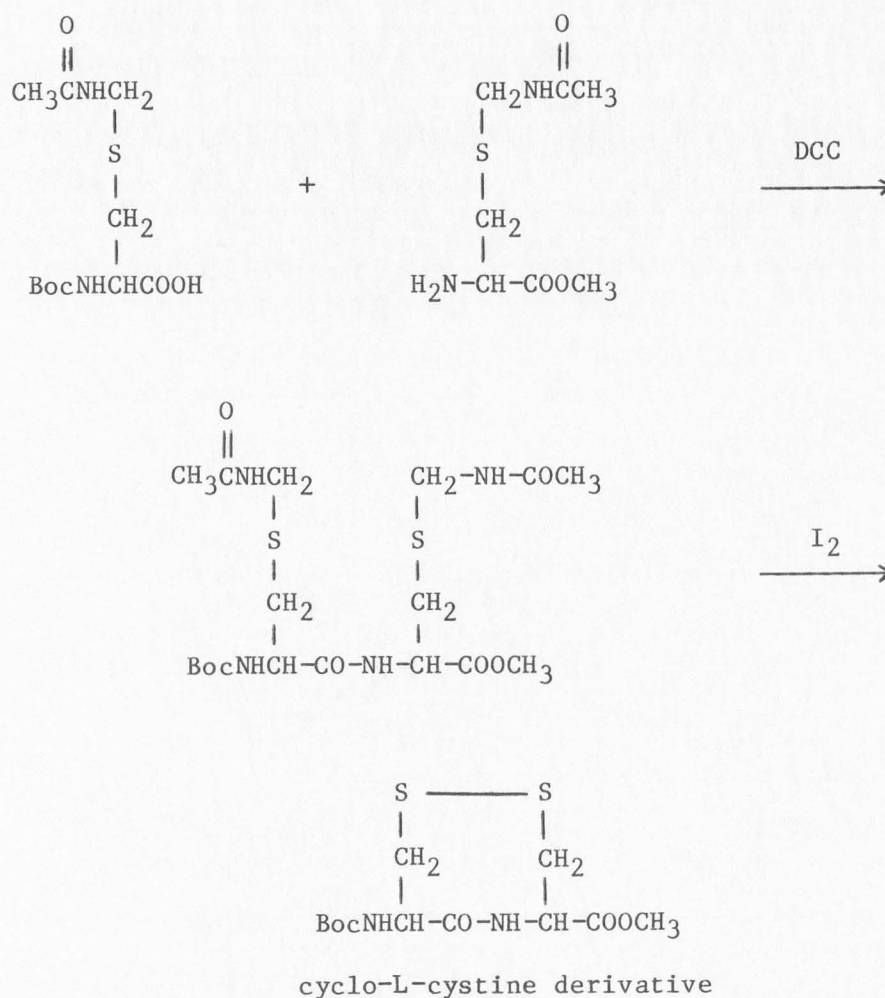
Synthesis of unsymmetrical cystine containing peptides

As in the case of lanthionines, several methods have been reported for the synthesis of unsymmetrical disulfides,⁷⁰⁻⁸⁰ where as relatively few are available for the synthesis of cystine containing peptides.

In 1973, Kamber⁸¹ synthesized various fragments of insulin containing a disulfide bridge. The fragmentation of sulfenyl thio carbonate with a thiol unit can be applied to the synthesis of unsymmetrical cystine peptides.

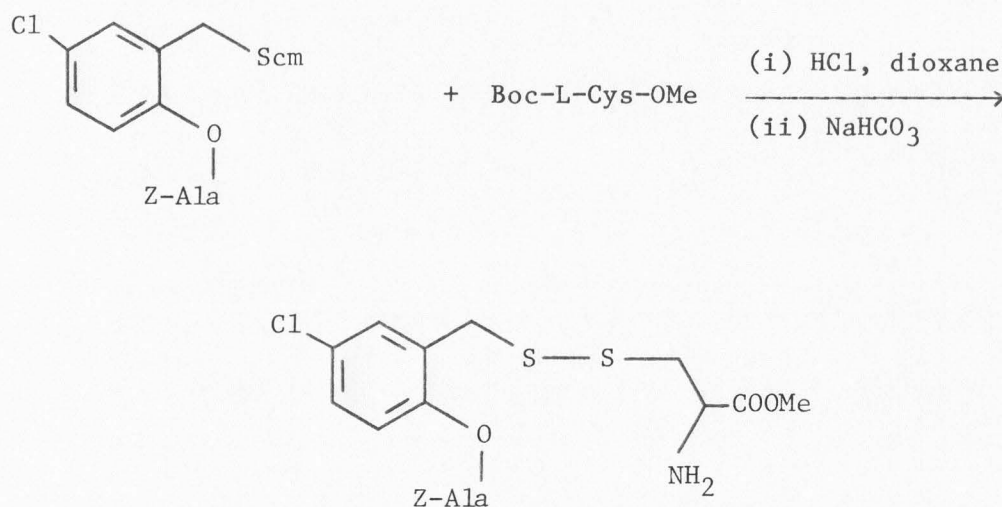


Kamber also reported⁸² the synthesis of cystine peptides by the reaction of S-acetamidomethyl cysteine peptides with iodine. Using this method, Kamber was able to synthesize cyclo-L-cystine, which, at that time, was unknown.

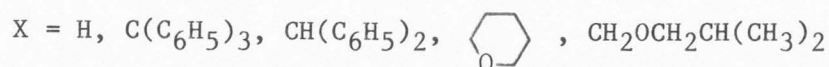
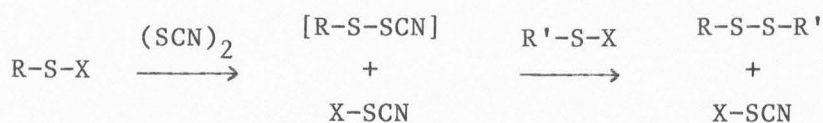


DCC: N,N'-dicyclohexylcarbodiimide

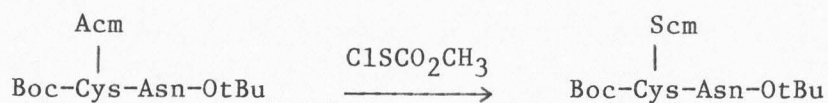
The reaction of L-cysteine esters with S-carbomethoxy derivatives to yield L-cysteine containing disulfides has been studied by Kemp and coworkers.⁸³

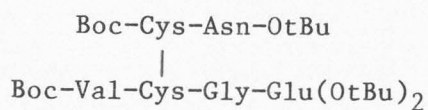
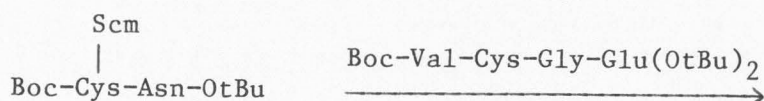
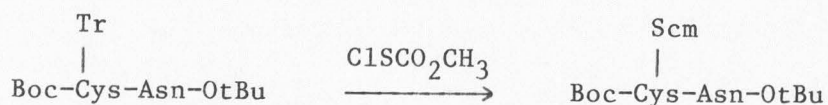


Hiskey and Ward⁸⁴ reported their studies on the synthesis of cystine peptides using sulfenyl thiocyanates. They applied this method to the synthesis of numerous cystine peptides with different amino acids, and observed no significant side reactions.



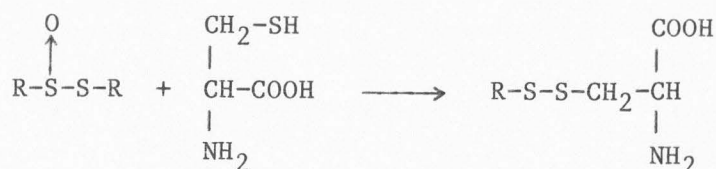
Hiskey and coworkers⁸⁵ also reported the synthesis of unsymmetrical cystine peptides using the S-carbomethoxy sulphenyl group as a labile intermediate for the selective conversion of a cysteine residue to a cystine.





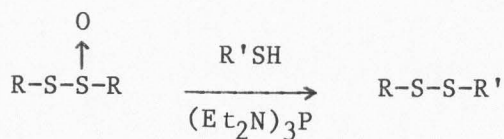
Acm: acetamidomethyl
 Tr: triphenylmethyl
 Scm: S-carbomethoxy
 tBu: tert-butyl

The reaction of thiolsulfinates with cysteine to yield unsymmetrical disulfides containing cysteine has been reported.⁸⁶

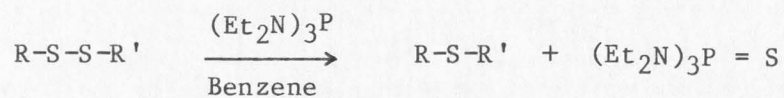


The method used for the synthesis of unsymmetrical lanthionines in this thesis is an approach based upon mechanistic consideration of the disulfide contraction reported by Harpp and Gleason.⁶⁵

An extension of the method of Schoberl,⁸⁶ involving the addition of a cysteine to a cystine sulfoxide, resulted in an unsymmetrical cystine.

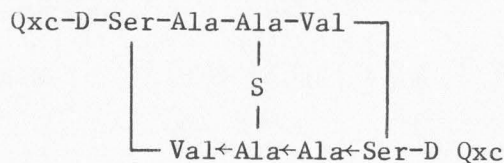


This unsymmetrical cystine was then selectively desulfurized with hexaethyl phosphorus triamide, used either in stoichiometric amount or in excess. These details are discussed later.



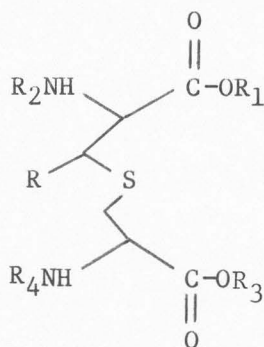
RESULTS AND DISCUSSION

The structure of the target molecule outlined for synthesis, the lanthionine analog of TANDEM, is as shown below, structure 4.



4

4 is a symmetrical bicyclic octadidepsipeptide, with a C_2 axis of symmetry through the sulphur atom. 4 differs from TANDEM in that the central bridging unit has one sulfur atom as compared to two in TANDEM, with the rest of the molecule being the same for both 4 and TANDEM. The central bridging unit of 4 is a lanthionine unit 5.



5: R = H
5a: R = SCH₃

In the previously reported synthesis of triostin A and TANDEM, the cyclic octapeptide is synthesized first and the central bridging unit incorporated in the penultimate step of the total synthesis. The

approach presented in this thesis is to start with a suitably protected lanthionine unit 5 and develop a peptide synthesis about the lanthionine unit. As part of a continuing program towards the total synthesis of echinomycin, the methods developed for 5 could then be applied to a preformed β -methylthio lanthionine unit 5a, which is the central bridging unit in echinomycin, thus leading to echinomycin. Hence the studies towards the synthesis of 4 would provide a model for the total synthesis of echinomycin.

The second aspect of interest in the synthesis of 4 is the possible manifestation of the binding properties of 4 to DNA. As mentioned before, TANDEM differs from the naturally occurring triostin A by the absence of four N-methyl groups on the cysteine and valine residues. TANDEM, however, exhibits opposite sequence specificity in its binding to DNA as compared to triostin A. It has been established that small changes in structure lead to significant difference in activity among the quinoxaline antibiotics. Hence, it would be interesting to compare the binding properties of 4 with TANDEM and triostin A. The central bridging unit of 4 has the same number of atoms as that in echinomycin. Thus, a comparison of the binding properties of 4 to those of echinomycin would be of considerable interest and biological significance.

The symmetry of the target molecule 4 prompted the design of the total synthesis of 4 starting with a symmetrical lanthionine. Attempted cyclization of the octapeptide synthesized, however, was unsuccessful. This led to the logical alternative to start the synthesis with an unsymmetrical lanthionine with appropriate protecting groups that would permit the cyclization leading to the bicyclic

octapeptide 4 to be effected in two separate steps, the first one leading to one half of the bicyclic system, with the second leading to bicyclic octapeptide 4.

The synthesis of a suitable lanthionine unit with four different and compatible protecting groups was found to be a challenging one. Different approaches to the synthesis of lanthionines had to be pursued. Hence, while the synthesis of two suitably protected lanthionines was accomplished, the subsequent syntheses leading to octapeptide lanthionine 4 as proposed, were not carried out.

Thus, a synthesis starting with a symmetrical lanthionine unit was designed, and as shown in Scheme 2.

Preparation of lanthionine 27 from
N,N'-di-t-butyloxycarbonyl-L-cystine
dimethyl ester

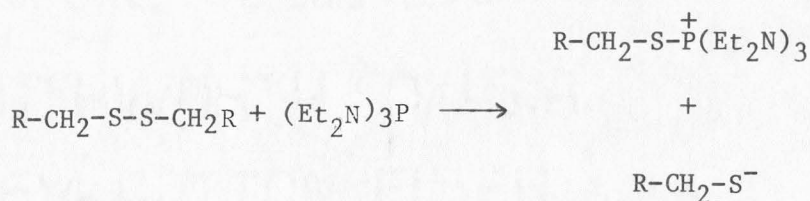
N,N'-Di-t-butyloxycarbonyl-L-cystine dimethyl ester 26 was prepared by the reaction of di-t-butyl-dicarbonate⁸⁷ with commercially available L-cystine dimethyl ester dihydrochloride, in N,N-dimethylformamide solution in the presence of triethylamine. This method of introducing the Boc-protecting group is more convenient than the previously used method using t-butyloxycarbonyl azide, as the latter has explosive tendencies. This method is also known to give higher yields. Compound ²⁶28, upon treatment with hexaethylphosphorus triamide in benzene solution, underwent a clean disulfide contraction to yield lanthionine 27 in 80% yield after chromatographic purification using the mpls with 20% acetone in hexane as the eluting solvent.

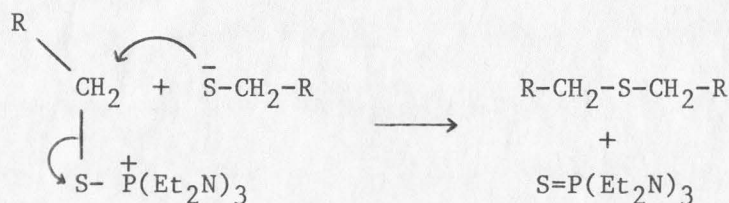
The ^1H NMR of 27 was identical to that of 26 with one exception. The cystine methylenes in 26 appear as a doublet at $\delta 3.2$, while those in 27 appear as a doublet at $\delta 3.0$. This is in accordance with the higher deshielding of the methylenes by two sulfur atoms in 26 as against one in 27. In fact, this was the only way by which we differentiated 26 and 27, other than elemental analysis, since they both have identical R_f values on tlc. It was observed that the conversion of 26 to 27 was essentially complete. A mixture of 26 and 27 would not be separable by silica gel chromatography because of their identical R_f values. C, H, N and S analysis data for 27 were in satisfactory agreement with the calculated values, thus confirming the desulfurization.

The mechanism of lanthionine contraction

The mechanism of this reaction, reported by Harpp and Gleason,⁶⁵ can be visualized as a two-step process. Step 1 is the nucleophilic attack of phosphorus on the sulfur, resulting in the formation of a positively charged phosphonium ion and a mercaptide anion. Step 2 is the attack of the mercaptide anion on the carbon α to the sulfur of the phosphonium ion, resulting in the formation of the lanthionine and phosphine sulphide.

Step 1



Step 2

Synthesis of and cyclization studies
of linear octapeptides 29 and 32

Compound 27 was hydrolyzed with sodium hydroxide in dioxane-water to yield the diacid in 86% yield. This diacid was coupled with dipeptide N-benzyloxycarbonyl-O-(N-Boc-L-valyl)-D-serine p-bromophenacyl ester 5 to yield hexapeptide 28. ^1H NMR spectrum of 28 showed the characteristic absorption doublet at δ 1.0 of the valine isopropyl methyls. An A_2B_2 quartet at δ 7.8 corresponding to the phenacyl aromatic was present. Elemental analysis data were in satisfactory agreement with the calculated values.

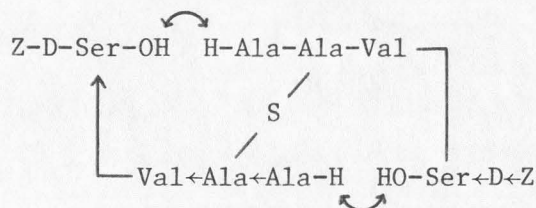
Treatment of 28 with trifluoroacetic acid, followed by coupling with N-t-butyloxycarbonyl-L-alanine led to 29. The structure of 29 was confirmed by the presence of the absorption peaks by the alanine methyls at δ 0.9 and satisfactory combustion analysis. The couplings to yield 28 and 29 were carried out in tetrahydrofuran using water soluble carbodiimide (EDC) and 1-hydroxybenzotriazole.

Deprotection of the amino groups with trifluoroacetic acid followed by reductive hydrolysis of the p-bromophenacyl ester groups with zinc and acetic acid led to octapeptide 30 with two free carboxyl and two free amino groups.

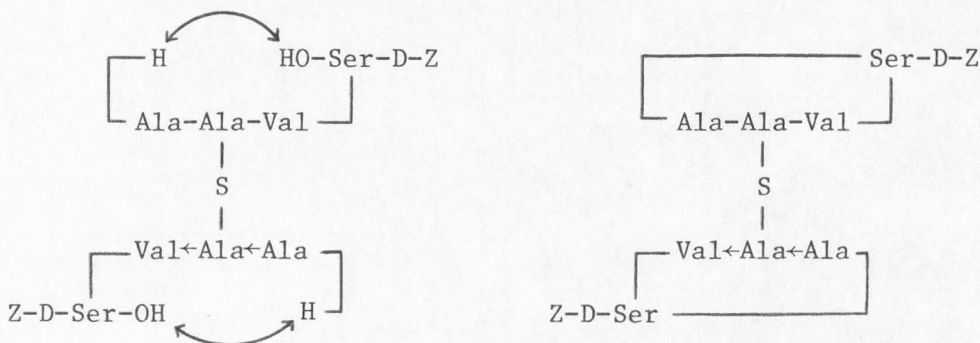
There were two potential problems in the cyclization of octapeptide 30 to yield 31. One was intermolecular coupling, where

Sequence
reversed, see
p. 75.

instead of cyclization, the product would be a mixture of polypeptides. This was minimized by performing the reaction under high dilution, which would facilitate only intramolecular coupling. The second potential problem was intramolecular coupling in the undesired mode, as illustrated below.



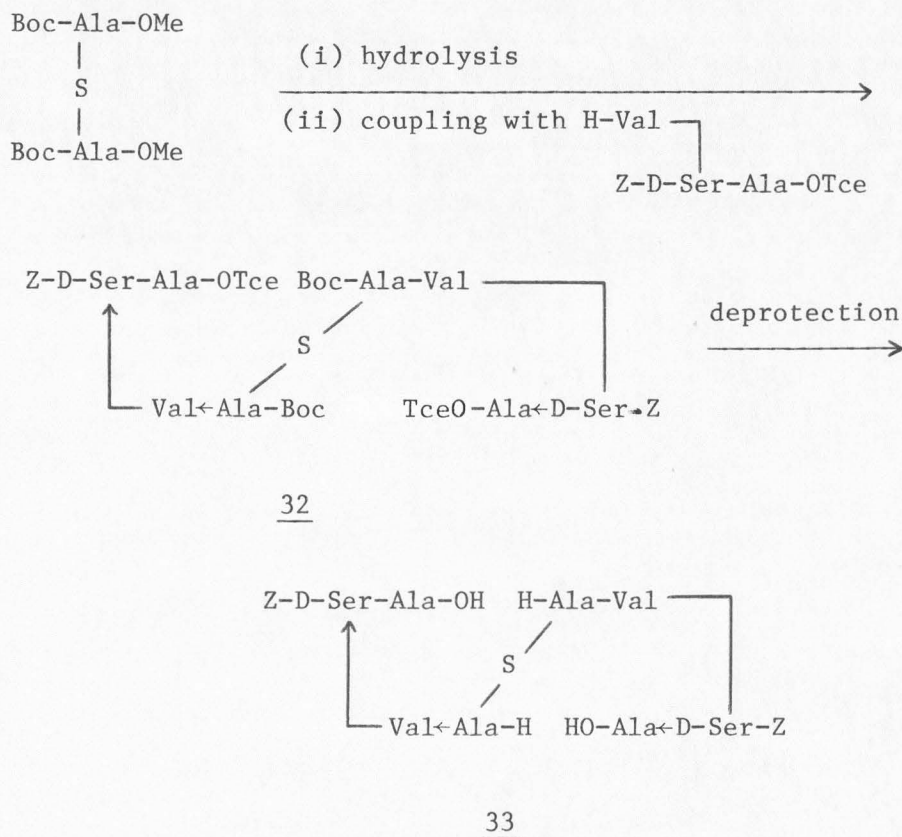
Desired mode of coupling



Undesired mode of coupling

The undesired mode of coupling involves the formation of a thirteen membered ring as against a seventeen membered ring to give the desired product. Since a 17-membered ring formation is favored over a 13-membered one,⁸⁸ it was presumed that the formation of the bicyclic octapeptide 31 would be favored. The products of attempted coupling of 30 were compared to an authentic sample with a disulfide bridge instead of a monosulfide, as it was assumed that they would both have the same Rf values on tlc. Tlc analysis showed the product of reaction to be a complex mixture. Attempts to isolate 31 were unsuccessful.

Octapeptide 32 was synthesized by coupling of lanthionine 27, after hydrolysis, with a previously reported²¹ tridepsipeptide N-benzyloxycarbonyl-0-(L-valyl)-D-seryl-L-alanine 2,2,2-trichloroethyl ester. The coupling was carried out in THF with EDC and N-hydroxysuccinimide.

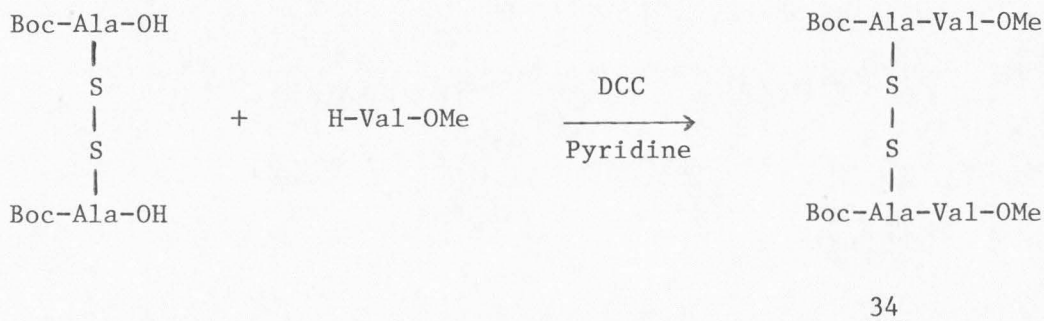


Removal of the Boc-groups upon treatment of 32 with trifluoroacetic acid, followed by reductive cleavage of the trichloroethyl ester functions with zinc in acetic acid, led to octapeptide 33. Attempted cyclization of 33 to 31 under conditions identical to those used in the cyclization of 30 to 31 resulted in a complex mixture of products and 31 could not be isolated. The cyclization of 30 to 31 involves amide bond formation between the C-terminal of the serine and the corresponding N-terminal of the alanine

units. In the case of 33, however, the amide bond would be formed between the C-terminal of the alanine moiety with the N-terminal of the lanthionine unit. Both modes of cyclization have been reported^{21,45} in the synthesis of cyclic octadepsipeptides without the central sulfur unit.

Studies on lanthionine contraction
of tetrapeptide 34, hexapeptide 35,
cyclooctapeptide 13 and TANDEM

In order to study the application of Harpp's method of disulfide contraction to other systems, tetrapeptide 34 was synthesized. N,N'-di-t-butyloxycarbonyl-L-cystine was coupled with the methyl ester of L-valine in pyridine using dicyclohexylcarbodiimide to yield 34.

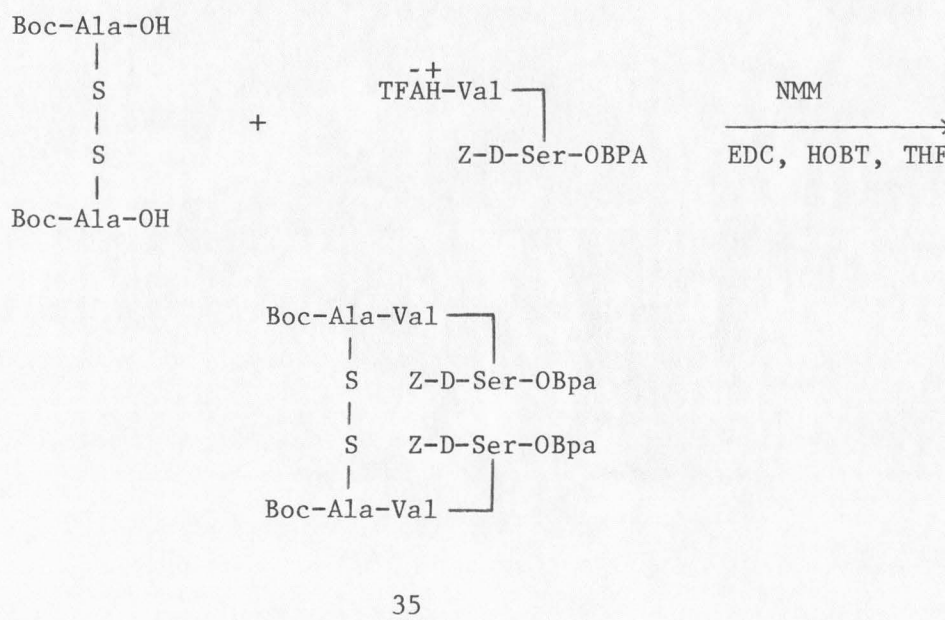


DCC: dicyclohexylcarbodiimide

¹H NMR of 34 showed the characteristic absorption peak for the valine isopropyl methyls as a doublet at δ 1.0.

Tetrapeptide 34 was treated with 1.2 eq of hexaethylphosphorus triamide in benzene solution. Upon purification of the reaction product on mplc, the corresponding lanthionine was obtained in 30% yield. The lanthionine showed the absorption peak of the cystine methylenes as a doublet at δ 3.0. C, H and N analysis were in agreement with the calculated values.

Hexapeptide 35 was synthesized as shown below.



NMM: N-methylmorpholine

EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl

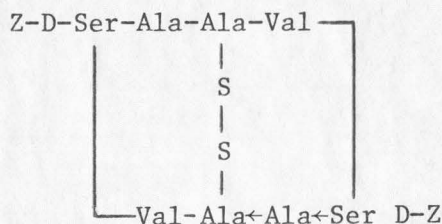
HOBT: 1-hydroxybenzotriazole

The structure of 35 was established by ^1H NMR data, which showed the aromatic protons of the p-bromophenacyl group resonating as an A_2B_2 quartet at δ 7.8. The absorption of the aromatics of the benzyloxycarbonyl groups appeared as a sharp singlet at δ 7.2. The valine isopropyl methyls resonated a doublet at δ 1.0. Elemental analysis for C, H, and N were in agreement with the calculated values.

Attempts to contract the hexapeptide 35 to the corresponding lanthionine were unsuccessful. Analysis of the products of reaction after treatment of 35 with hexethylphosphorus triamide indicated that the desired lanthionine was not obtained, and starting material was recovered.

A contraction of the cyclic octapeptide 13 shown below, which is a known intermediate prepared in the synthesis of TANDEM²¹, was attempted

using the above contraction procedure. The products of reaction were separated and isolated by preparative tlc. 360 MHz ^1H NMR spectral data

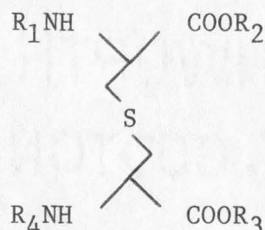


13

of the product showed no shift in the position in the cystine methylenes before and after the attempted contraction. This was further confirmed by the fact that the ^1H NMR of a mixture of 13 and the isolated contraction product, a mixture which was homogeneous on tlc, was identical to the ^1H NMR of 13. Hence, the contraction could not be effected. The reaction was carried out again, with a slight modification. Instead of stirring at room temperature for an hour, the reaction mixture was stirred at room temperature overnight. The results of this reaction were the same as before, the disulfide was unaffected. An attempt to contract TANDEM itself was also unsuccessful.

The unsymmetrical approach to the synthesis of 4

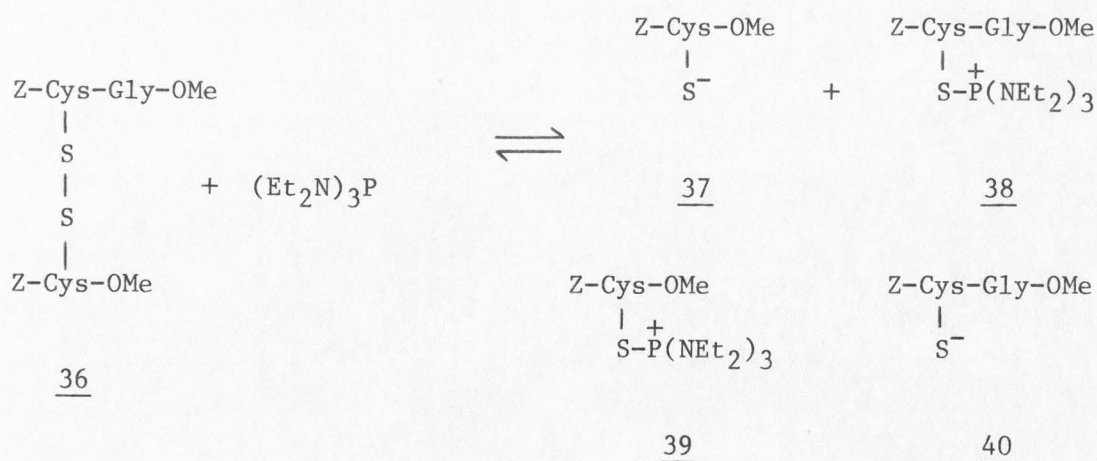
The failure of the symmetrical approach to the synthesis of 4 thus led to a logical alternative. An unsymmetrical lanthionine with four different protecting groups, as shown below, would, in principle, permit the cyclization of one half of the bicyclic octadepsipeptide followed by the other in two separate steps.



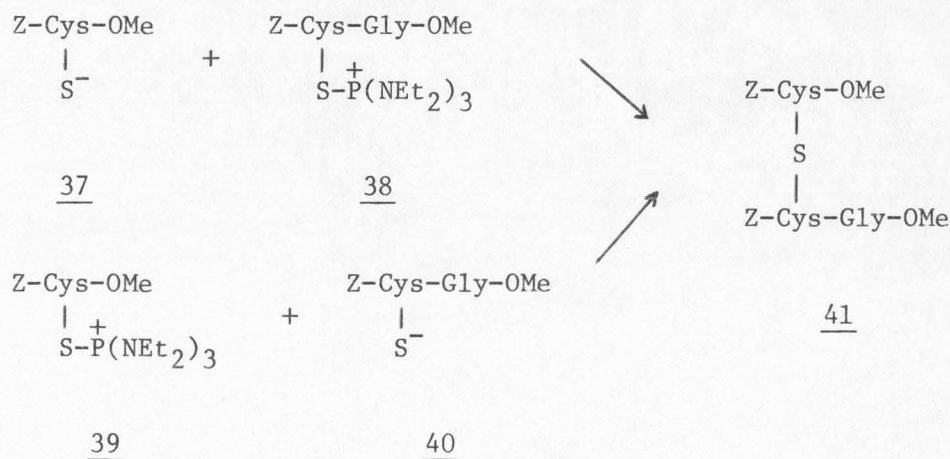
As mentioned before, there have been few methods reported for the synthesis of lanthionine. The synthesis of the above lanthionine posed an additional challenge in that the four protecting groups, R_1 , R_2 , R_3 and R_4 , had to be compatible within themselves as well as with the two depsipeptide ester functions and other protecting groups present in subsequent peptide intermediates.

Contraction of unsymmetrical disulfides

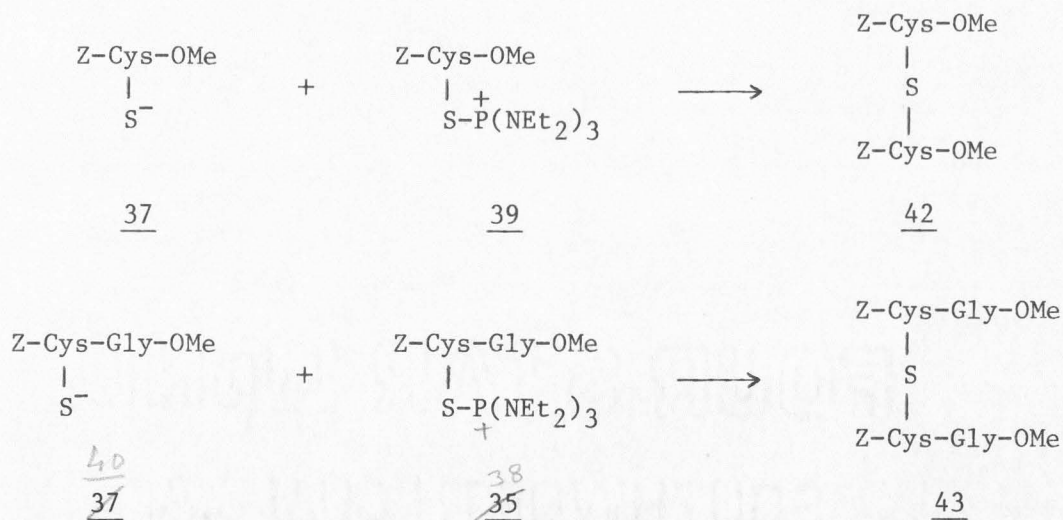
In an attempt to synthesize an unsymmetrical lanthionine, Harpp and Gleason⁶⁵ subjected peptide 36 to treatment with hexaethylphosphorus triamide. Contrary to the expected peptide 41 (see p. 39), only peptide 45 (see p. 40) was isolated. To explain these results, an equilibrium, with the intermediates as shown below, was proposed. (The notations used are incorrect in peptide terminology, but have been used for simplicity, as used by Harpp and Gleason.)



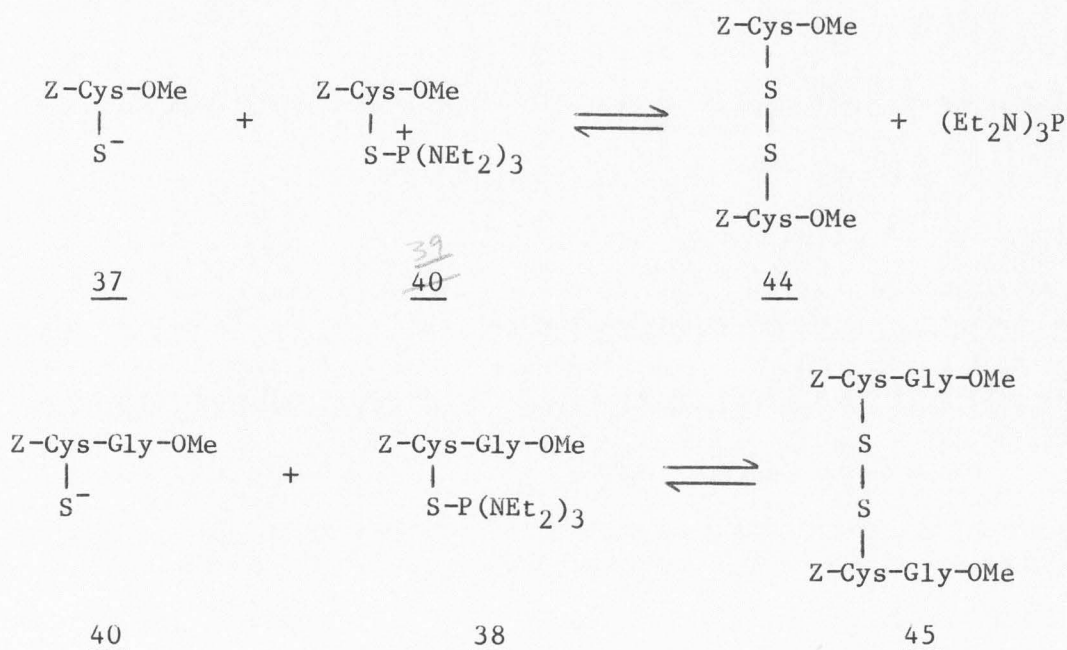
Treatment of 36 with hexaethylphosphorus triamide could result in the formation of two sets of charged intermediate 37 and 38, and 39 and 40. According to the accepted mechanism for the formation of lanthionine, reaction of either set should yield desired lanthionine 41.



However, reaction of 37 with 39 could lead to symmetrical lanthionine 42; analogously, reaction of 38 with 40 could lead to symmetrical lanthionine 43.

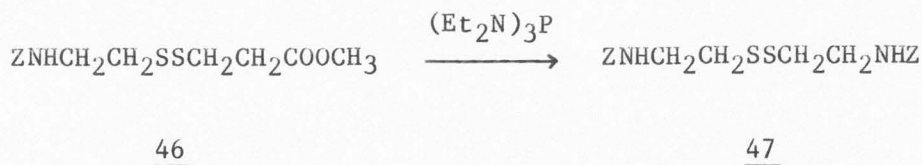


Intermediates 37 and 40 could also be involved in equilibria leading to the corresponding symmetrical disulfides 44 and 45.



The isolation of 45 was attributed to its extreme insolubility in the solvent system used. The major products of this reaction were 45 and 42.

The attempted contraction of another unsymmetrical disulfide 46 also resulted in the formation of symmetrical disulfide 47 as the major product of reaction.



Symmetrical disulfide 47 was obtained in 88% yield. In this case, the formation of tris(diethylamino)phosphine sulfide was not observed. This observation led to the conclusion that equilibration of disulfides occurs faster than desulfurization. The disproportionation of unsymmetrical disulfides into symmetrical disulfides is known to be a common side reaction in the synthesis of unsymmetrical disulfides.⁷⁴

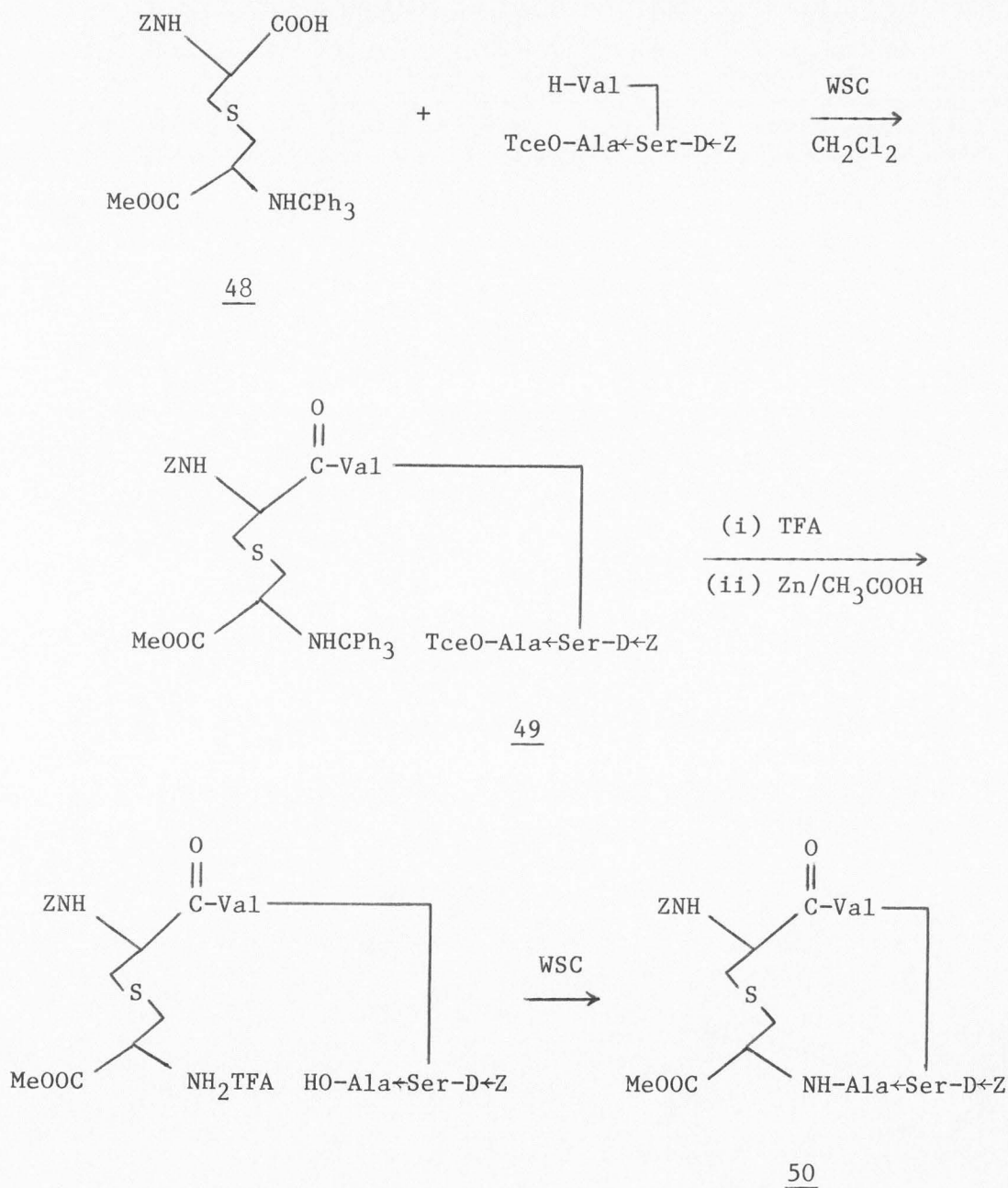
Another limitation of the above disulfide contraction method was that the C-terminal of the cystine had to be protected, since phosphines are known to react with carboxylic acids.⁷⁵

Thus, while the formation of unsymmetrical lanthionines did not occur in yields of synthetic utility because of the possible equilibria already discussed, the contraction of symmetrical tetrapeptide 34 in low yields as compared to that of 26 and no contraction at all of hexapeptide 35 was indicative of the fact that this reaction may be sensitive to steric effects. In comparison to 26, the valine isopropyl group in tetrapeptide 34 could sterically hinder the nucleophilic attack of the phosphine phosphorus on the sulfur, either because of its size or due to a conformational effect. The same could be true for hexapeptide 35, but to a larger extent, thus resulting in no contraction at all to yield the corresponding lanthione. This could also be the possible reason for the lack of reactivity of TANDEM and the cyclic octapeptide intermediate, towards hexaethylphosphorus triamide. In the latter case, the ring conformation in the bicyclic structure could be such that the sulfur-sulfur bond is effectively shielded from nucleophilic attack by the phosphine phosphorus. Hence, it was necessary to look into other possible synthetic routes to the desired unsymmetrical lanthionine.

Synthesis of lanthionine by
nucleophilic displacement of
 β -chloro-L-alanine

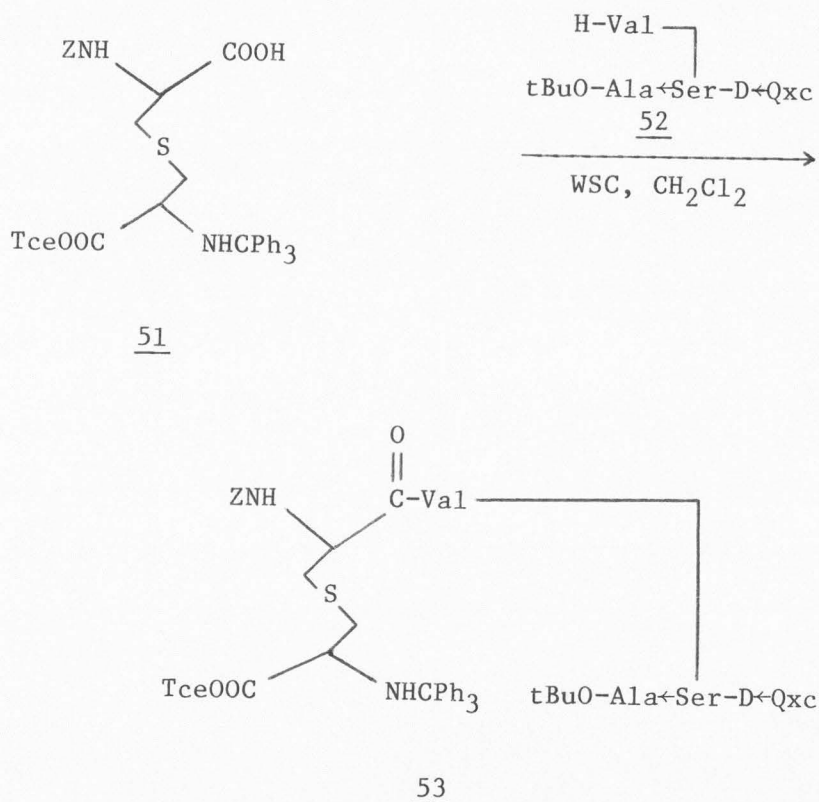
Photaki and coworkers⁶⁶ reported the synthesis of unsymmetrical lanthionine 48 by the displacement of a halide ion with a thio anion. The rationale for the synthesis of 48 was two fold. One was to carry

out model studies on the cyclization leading to a cyclic pentapeptide equivalent to one half of the target bicyclic octapeptide 4. The cyclic pentapeptide 50 exists with the same number of carbon atoms in the ring system as in one half of the symmetrical bicyclic octapeptide 4. As already mentioned, the proposed synthesis of pentapeptide 50 is shown schematically below.



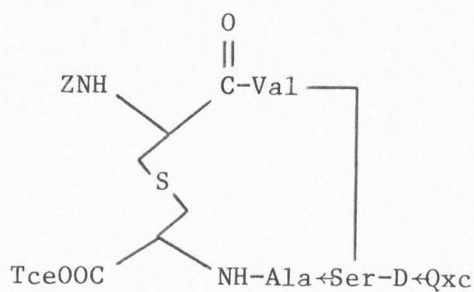
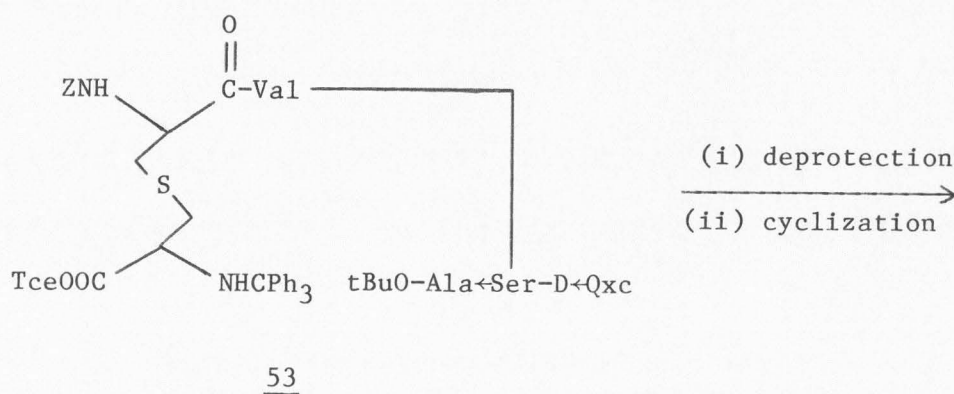
Coupling of lanthionine 48 with previously synthesized²¹ tridepsipeptide using water soluble carbodiimide would yield pentapeptide 49. Deprotection of the lanthionine amine followed by reductive cleavage of the 2,2,2-trichloroethyl ester with zinc in acetic acid would set up 49 for a cyclization leading to cyclic pentapeptide 50.

The second aspect of interest in the synthesis of 48 and 50 was the potential application of this system, with appropriate modifications, to the proposed synthesis of cyclic pentapeptide 54, shown schematically below.

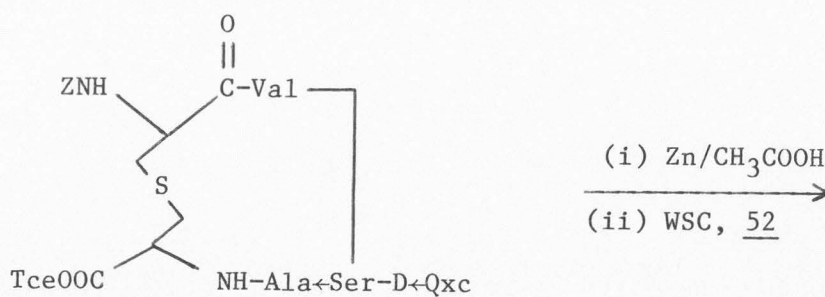


Lanthionine 51, synthesized along the same lines as lanthionine 48, upon coupling with tridepsipeptide 52 would lead to linear pentapeptide 53. The synthesis of 52 has been reported by Shin and

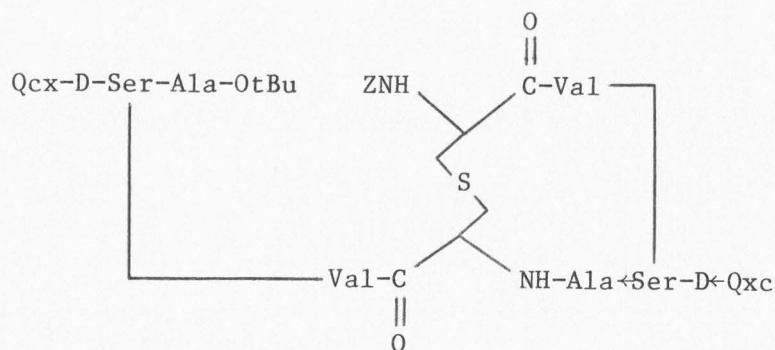
coworkers.⁴⁹ Deprotection of 53, followed by cyclization, would then give cyclic pentapeptide 54.



Treatment of 54 with zinc in acetic acid followed by coupling with tridepsipeptide 52 would lead to linear octapeptide 55.

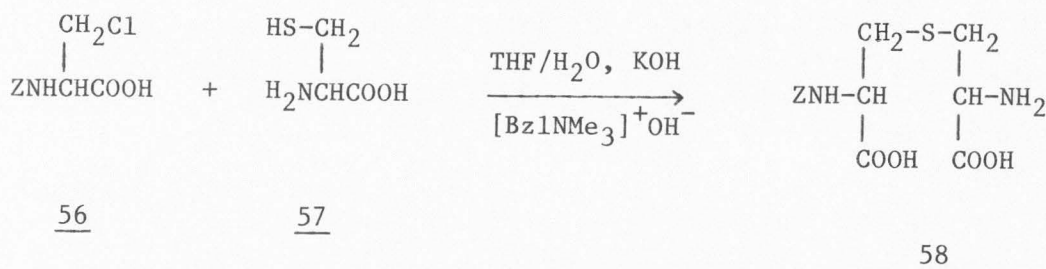


(i) Zn/CH₃COOH
 (ii) WSC, 52

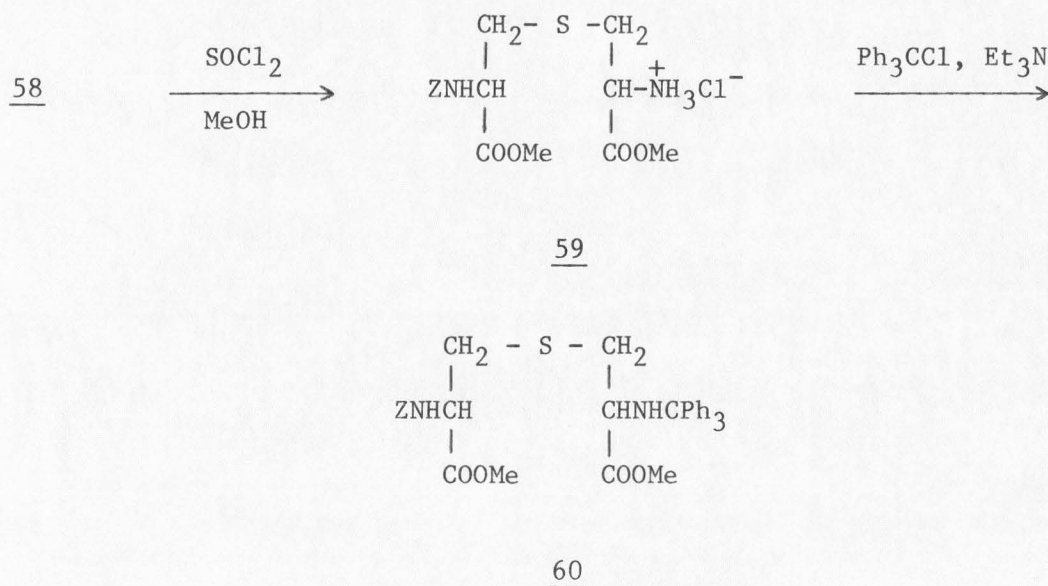


Treatment of octapeptide 55 with HBr in acetic acid⁸⁹ would deprotect the amine and the carboxyl functions. Cyclization would thus lead to bicyclic octadepsipeptide 4.

The syntheses towards lanthionine 48 were carried out by the procedure of Photaki and coworkers⁶⁶ as per the sequence of reactions outlined below.



N-Benzyloxycarbonyl- β -chloro-L-alanine 56, upon reaction with L-cysteine 57, in the presence of aqueous base under phase transfer conditions, gave lanthionine 58. The diacid 58 was esterified under standard conditions with thionyl chloride and methanol to yield dimethyl ester 59. Treatment of 59 with triphenylmethyl chloride and triethylamine led to the N-tritylated lanthionine 60.



^1H NMR of 60 showed two peaks corresponding to the two methyl esters, at δ 3.2 and δ 3.7. It was inferred that the ester methyl on the carbon β to the tritylated amine group is oriented such that it lies in the shielding zone of phenyl rings of the trityl group, and hence resonates upfield. Hydrolysis of 60 under alkaline conditions should lead to mono acid 48, since the trityl group, by virtue of its size, sterically shields the ester of the neighboring carboxyl from hydrolysis.⁹⁰

Attempted hydrolysis of 60 with aqueous NaOH in acetone resulted in a complex mixture of products. The components of this mixture were separated by mplc. ^1H NMR analysis indicated that the desired lanthionine mono acid 45 was not obtained. ^1H NMR of one component was consistent for the mono acid with loss of the trityl group. The trityl group probably was lost upon chromatography. Thus, in our hands the method of Photaki and coworkers⁶⁶ was found not to be a viable route for the synthesis of 51, particularly in view of the fact that 51 would

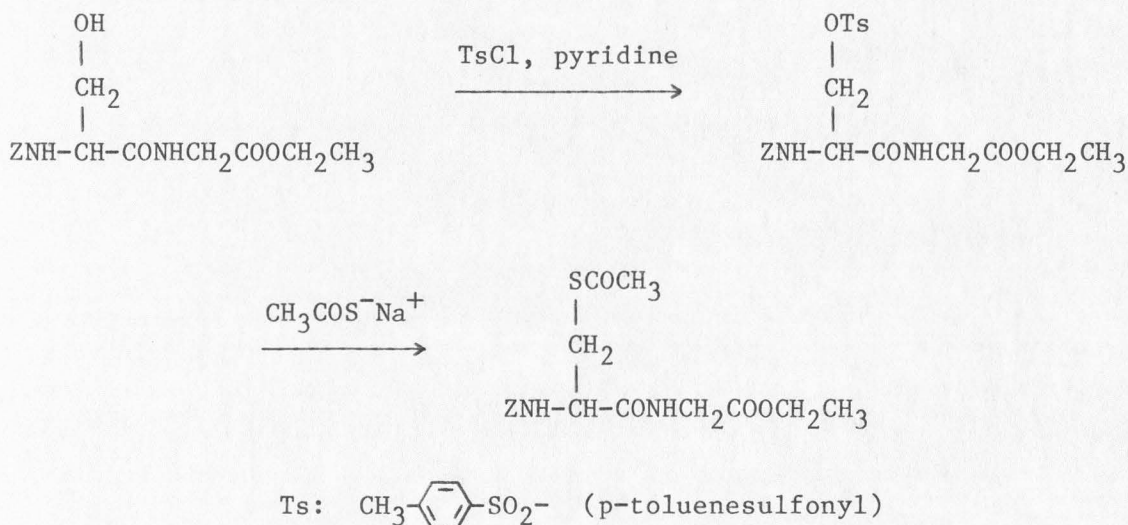
be the starting compound in the total synthesis, involving several subsequent steps, of octapeptide lanthionine 4.

This method was also found to be tedious in the synthesis of N-benzyloxycarbonyl- β -chloro-L-alanine itself. An interesting observation was made during the synthesis of the protected amino acid. Commercially available β -chloro-L-alanine hydrochloride, upon treatment with benzyl chloroformate and NaHCO_3 in aqueous solution, standard conditions for the introduction of the benzyloxycarbonyl group, led to the N-benzyloxycarbonyl- β -chloro-L-alanine in yields ranging from 20-40%. Upon increased dilution of the reaction mixture, by the addition of water, the N-protected amino acid was obtained in 80% yield. The reaction of N-benzyloxycarbonyl- β -chloro-L-alanine with L-cysteine to yield 58, the first step in the reaction sequence leading to lanthionine 48, was found to be a highly sensitive one, with a strong dependence on the pH of the reaction mixture for the product to separate out of the aqueous solution as a white solid.

The use of cesium carbonate as base to generate a thiol anion under mild conditions, in the alkylation of halides with the thiol anion, has been reported.⁹¹ Attempts to alkylate N-benzyloxycarbonyl- β -chloro-L-alanine with N-t-butyloxycarbonyl-L-cysteine methyl ester and cesium carbonate, in dimethylformamide solution, resulted in a complex mixture of products, with no desired lanthionine monoester obtained.

Synthesis of lanthionine by
displacement of O-activated
serine derivatives with cysteine

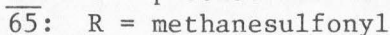
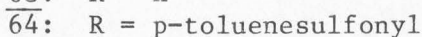
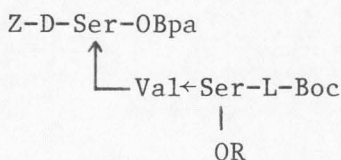
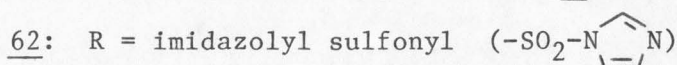
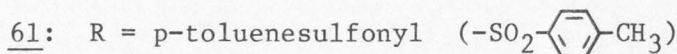
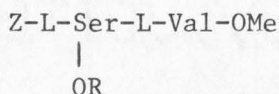
Wilchek and coworkers⁶⁸ converted the L-serine residue in peptides to an L-cysteine residue by tosylation of the serine hydroxyl, followed by nucleophilic displacement with a thio anion.



This method was applied by the above workers⁶⁸ to various L-serine containing peptides using several thio anions such as those of triphenylthiocarbinol, benzyl mercaptan, and 2-mercaptoacetic acid. It was observed that when the carboxy terminal of the tosylated serine existed as an amide function, the displacement of the tosylate with a thio anion led to an optically active cysteinyl peptide. However, when the same reaction was carried out on a serine peptide in which the carboxy terminal of the tosylated serine existed as an ester, the resulting cysteinyl peptide was optically inactive. As already mentioned, this observation was attributed to the fact that in the case of the ester, an elimination occurs to yield an α,β -unsaturated ester. The mercaptide anion then adds 1,4- to the α,β -unsaturated ester to yield racemic cysteinyl peptide.

Hence the adaptation of this method to the synthesis of a suitable optically active lanthionine or cysteinyl peptide necessitated the carboxy terminal of the tosylated serine to be incorporated as an amide function. In order to carry out studies on the synthesis of cysteinyl peptides by the displacement of activated serine hydroxyl with suitable

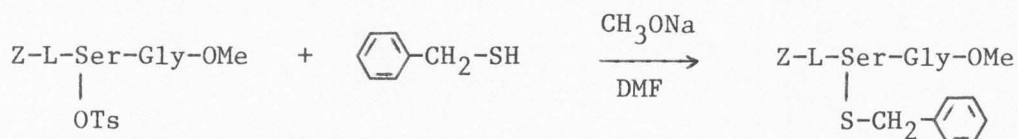
thio anions, the following compounds 61 to 65 were outlined for synthesis.



N-Benzyloxycarbonyl-L-serine was coupled with L-valine methyl ester using dicyclohexylcarbodiimide to yield dipeptide N-benzyloxycarbonyl-L-seryl-L-valine methyl ester in 71% yield. The dipeptide was a crystalline compound with the 60 MHz ^1H NMR spectrum showing the absorption peaks of the valine isopropyl methyls as a doublet at δ 0.9. The benzyloxycarbonyl group protons resonated as singlets at δ 5.2 and δ 7.5 for the benzylic methylenes and the aromatics, respectively. Treatment of the dipeptide with p-toluenesulfonyl chloride in pyridine led to the tosylated dipeptide 61 in 60% yield after purification by flash chromatography. ^1H NMR of 61 showed a quartet at δ 7.8 characteristic of the aromatics of the p-toluenesulfonyl groups overlapping with a singlet at δ 7.5, the absorption of the benzyloxycarbonyl aromatic protons. Dipeptide tosylate 61 was then reacted with benzylmercaptan and triethylamine.

The two products of reaction were the starting dipeptide tosylate 61 and another compound identified by 60 MHz ^1H NMR spectral analysis to be dibenzyl disulfide.

Wilchek and coworkers⁶⁸ reported the displacement of the tosylated hydroxyl group of serine in dipeptide N-benzyloxycarbonyl-L-seryl-glycine ethyl ester with benzyl mercaptide to yield the corresponding cysteinyl peptide. This dipeptide tosylate was synthesized by the same method used in the synthesis of 61. Reaction of the dipeptide tosylate with benzyl mercaptan and sodium methoxide in DMF solution did lead to the corresponding cysteine peptide.



Reaction of 61 with benzyl mercaptan under the same conditions, however, resulted in recovery of starting material and no desired cysteinyl peptide was obtained.

Thus it was concluded that the valine isopropyl group sterically hinders the nucleophilic displacement by the mercaptide anion. This could also be a conformation effect, predominant when the amino acid forming the peptide bond at the C-terminal of the tosylated serine is valine.

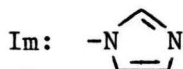
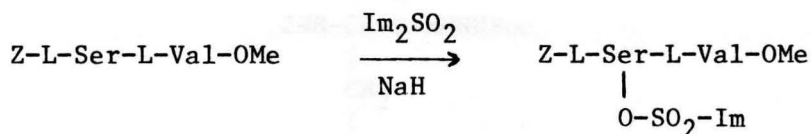
Tripeptide 63 was synthesized by coupling of dipeptide N-benzyloxycarbonyl-O-(N-t-butyloxycarbonyl-L-valyl)-D-serine p-bromophenacyl ester,⁴⁵ after deprotection of the valine amine terminal with trifluoroacetic acid, with N-t-butyloxycarbonyl-L-serine and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in 98% yield. 60 MHz ^1H NMR of 63 showed the characteristic absorption signal for the

Boc-methyls at δ 1.5. Treatment of 60 with 1.1 equivalents of p-toluenesulfonyl chloride in pyridine led to a product mixture, which from tlc analysis indicated that mainly starting tripeptide 63 was present. Attempts to synthesize 64 with an excess of p-toluenesulfonyl chloride led to identical results.

These results prompted the synthesis of tripeptide mesylate 65, since the methanesulfonyl group is smaller in size as compared to the p-toluenesulfonyl group. Reaction of tripeptide 63 with methane sulfonyl chloride in pyridine led to 65 in 96% yield. The 60 MHz ^1H NMR of 65 showed the absorption peak for the sulfonyl methyl as a sharp singlet at δ 3.0. Attempted displacement of the mesylate 65 with benzylmercaptan in the presence of sodium methoxide in dimethyl formamide solution resulted in recovery of starting materials as indicated by tlc analysis.

Hannessian and Vatele⁹² reported the imidazolyl sulfonate group to have some unique advantages over the tosylate and mesylate in their studies on nucleophilic displacement of the hydroxyl groups in carbohydrate systems. The imidazolyl sulfonate group was found to work better in the case of sterically hindered hydroxyls and also to minimize elimination reactions.

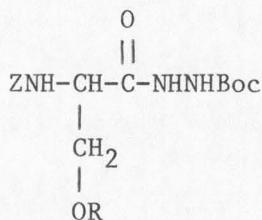
According to the method of Hannessian and Vatele,⁹² compound 62 was synthesized by reaction of dipeptide N-benzyloxycarbonyl-L-seryl-L-valine methyl ester with 1,1'-sulfuryl diimidazole⁹³ and sodium hydride in dimethylformamide solution. ^1H NMR of 62 showed the resonance of the imidazole protons at δ 7-8.2. Treatment of 62 with benzyl mercaptan and sodium methoxide in dimethylformamide resulted in



recovery of starting material with no desired cysteinyl peptide being formed, as indicated by tlc analysis.

Thus, the synthesis of a suitable lanthionine or a suitable cysteinyl peptide by nucleophilic displacement of a tosylated serine with a thiol had two constraints on the tosylated serine molecule. The C-terminus of the tosylated serine had to exist as an amide function. An L-valine residue at this terminus, which would be required for the synthesis of target molecule 4, was unsuitable because of its apparent steric hindrance to nucleophilic displacement of the tosylated serine. These two constraints together prompted the synthesis of the serine derivative 66, with a N-t-butyloxycarbonyl hydrazide function at the C-terminal. The Boc hydrazide function can be converted to an azide⁹⁴ and directly used for further peptide coupling. The use of azides in couplings of penta- and octapeptides has been reported.⁹⁵

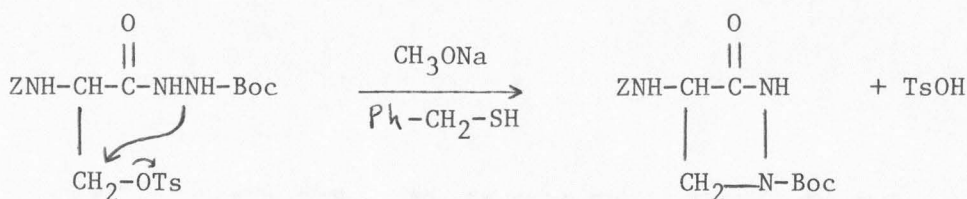
66 was synthesized in quantitative yield by treatment of N-benzyloxycarbonyl-L-serine with commercially available N-t-butyloxycarbonyl hydrazide and N,N'-dicyclohexylcarbodiimide. ¹H NMR of 66 showed an absorption signal for the Boc-methyls as a sharp singlet at δ 1.5. Tosylation of 66 with p-toluenesulfonyl chloride in pyridine led to tosylate 67.



- 66: R = H
67: R = p-toluenesulfonyl
68: R = imidazolylsulfonyl

The absorption signals for the p-toluenesulfonyl aromatics in the ^1H NMR spectrum of 67 appeared as a quartet at δ 7.8 overlapped by the absorption peak of the benzyloxycarbonyl aromatics at δ 7.4. The serine methylenes resonated as a multiplet at δ 4.4.

Attempted displacement of tosylate 67 with benzyl mercaptan and sodium methoxide in dimethyl formamide led to a product, the ^1H NMR of which was consistent with a cyclic intramolecular displacement product.



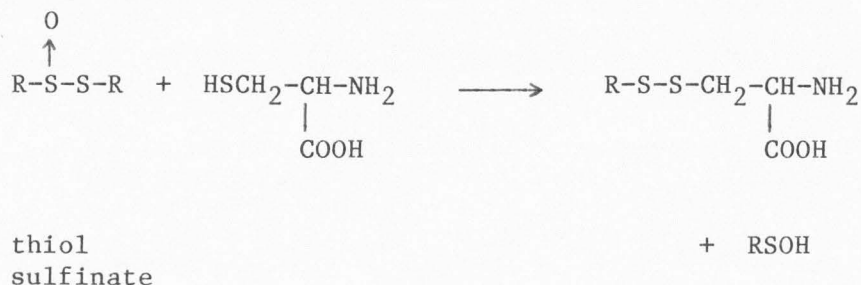
The absorption signals for the p-toluenesulfonyl aromatics were missing in the ^1H NMR spectrum of the product. The serine methylene absorption was shifted upfield from δ 4.4 in 67 to δ 3.8 in the 5-membered cyclic product.

The susceptibility of tosylate 67 to undergo intramolecular displacement of the tosyl group prompted the synthesis of imidazolyl sulfonate 68 under conditions which did not utilize the strong base sodium hydride. According to an alternate method reported by Hannessian and Vatele,⁹² 66 was treated with an excess of imidazole and

sulfuryl chloride. The product of this reaction was identical to that obtained in the attempted alkylation of 66. Apparently, the imidazolyl sulfonate first formed, undergoes a subsequent intramolecular displacement to yield the cyclic product.

Synthesis of unsymmetrical
lanthionines 76 and 80
using thiolsulfonates

The synthesis of unsymmetrical disulfides containing a cysteine unit by the reaction of thiolsulfonates with cysteine has been reported.⁸⁶ Field and Khim,⁹⁶ and Block and O'Connor⁹⁷ reported synthetic routes to thiolsulfonates from the corresponding disulfides.

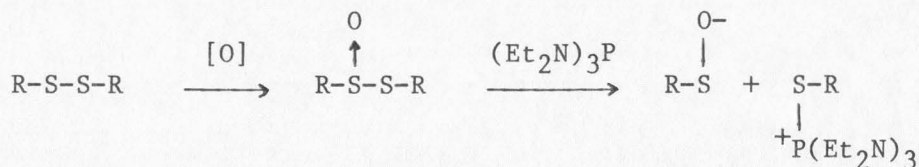


Boduszek and Kice⁹⁸ reported the general reaction of a nucleophile with a thiolsulfonate to be as follows.

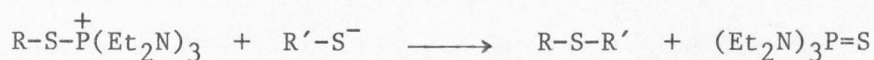


Thus, the two sulfur atoms in a disulfide or a cystine peptide could be distinguished towards nucleophilic attack by converting the disulfide to the corresponding thiol sulfonate. As already discussed, the first step in the method of Harpp and Gleason⁶⁵ for the synthesis of symmetrical lanthionines is a nucleophilic attack of the phosphine phosphorus on the sulfur of the disulfide. Then, thiol sulfonate, upon

treatment with hexaethylphosphorus triamide should yield the charged intermediates schematically shown below.

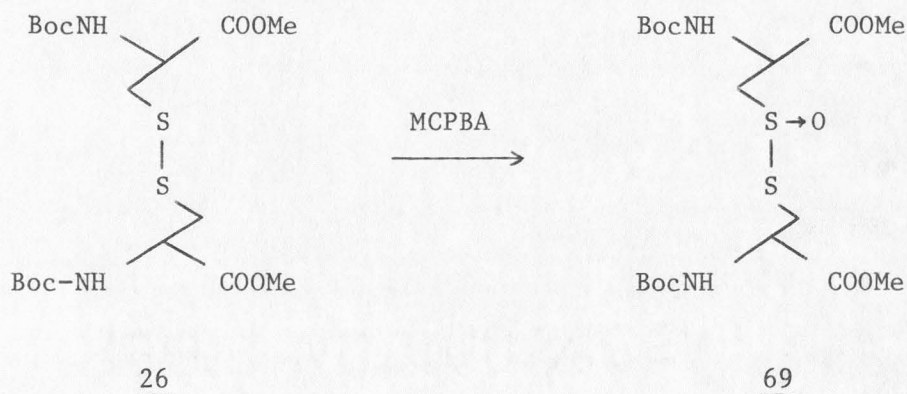


The sulfenic acid formed should not interfere with the reaction of a thiol anion with the positively charged phosphonium intermediate. The thiol anion and the phosphonium intermediate should react to yield a lanthionine, as discussed previously.

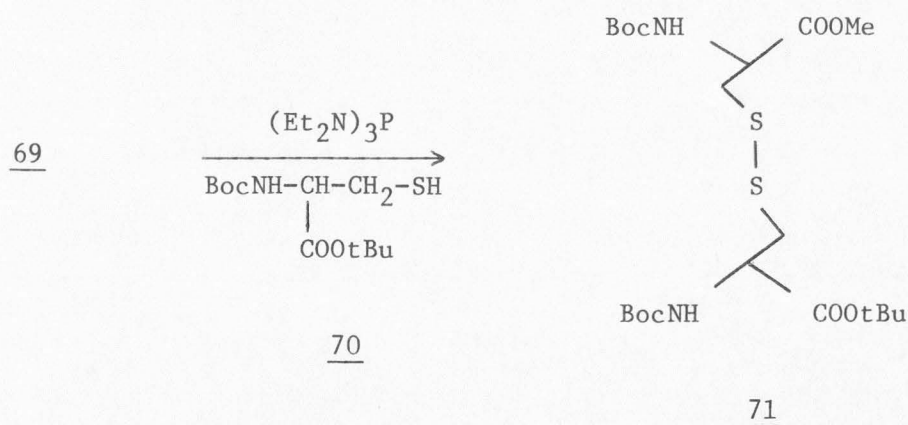


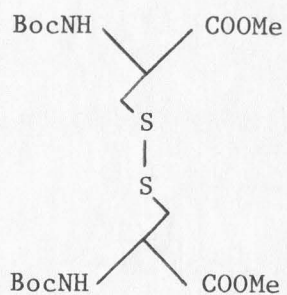
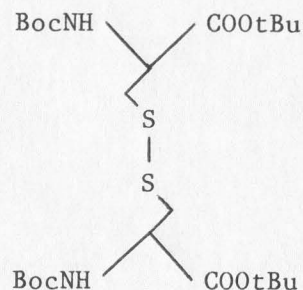
Thus a symmetrical and suitably protected cystine can, in principle, be converted to an unsymmetrical lanthionine according to the above scheme.

Preliminary studies towards synthesis of a suitably protected lanthionine via an appropriate thiolsulfinate were carried out by the synthesis of thiolsulfinate 69. N,N'-di-tert-butyloxycarbonyl-L-cystine di-methyl ester 26 was converted to the corresponding thiol sulfinate 69, according to the method of Wolfe and coworkers,⁹⁹ by treatment with m-chloroperbenzoic acid in methylene chloride.

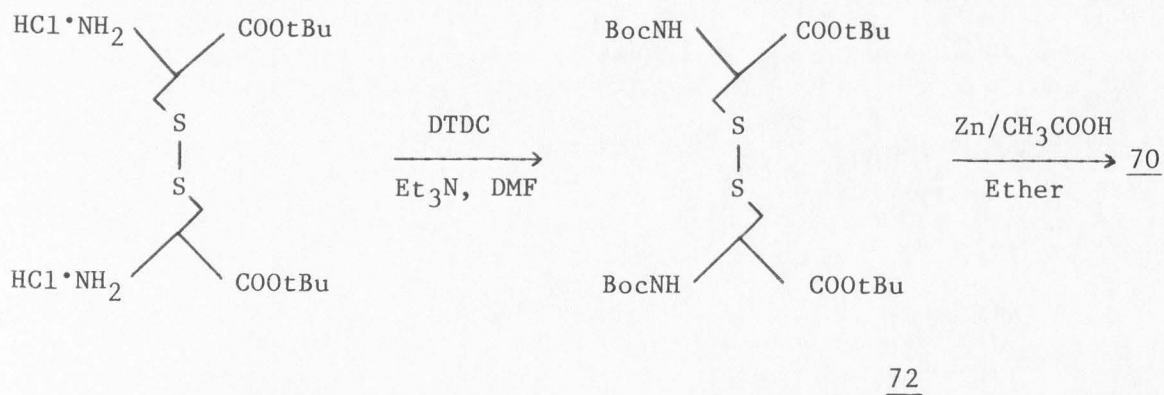


69 was reacted with one equivalent of hexaethylphosphorus triamide and N-t-butyloxycarbonyl-L-cysteine t-butyl ester (70) in benzene solution. The reaction mixture was chromatographed on the mplc and the fractions separated and analyzed by tlc and 60 MHz ^1H NMR. The major product of the reaction was disulfide 71 isolated in 50% yield. ^1H NMR of 71 showed the absorption peaks for the cystine methylenes as a doublet at δ 3.2, thus confirming the disulfide. Small amounts of symmetrical disulfides 26 and 72 were identified by tlc and ^1H NMR.



2672

Cysteine 70 was synthesized by reductive cleavage of cystine 72 with zinc and 5% acetic acid in ether in near quantitative yields. 72 itself was synthesized by treatment of L-cystine di-t-butyl ester dihydrochloride¹⁰⁰ with di-t-butyl dicarbonate and triethylamine in dimethylformamide solution.

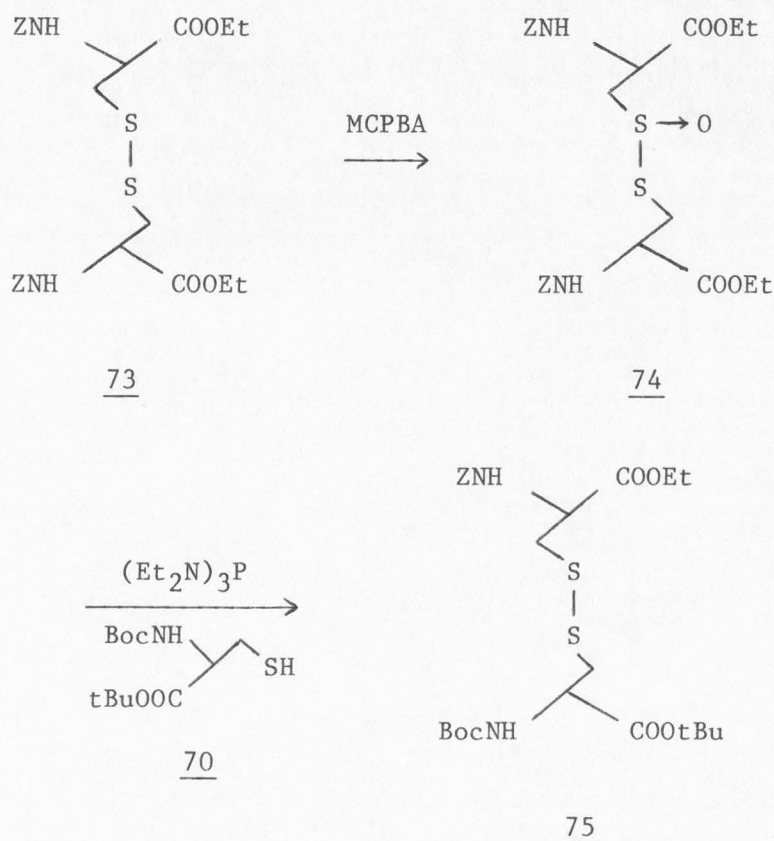


DTDC: Di-t-butyl dicarbonate

The chemistry, reactions and synthetic applications of thiol-sulfinates have been well documented.¹⁰¹⁻¹⁰³ To the best of our knowledge, this is the first example of the use of a thiol-sulfinate in the synthesis of a cystine or cystine containing peptide.

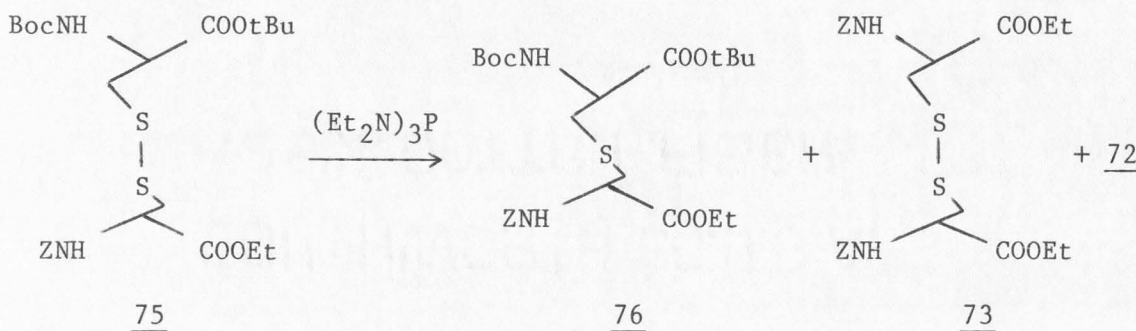
The synthesis of unsymmetrical cystine 75 was carried out with the same sequence of reactions, starting with a different thiosulfinate 74.

N,N'-Di-benzyloxycarbonyl-L-cystine diethyl ester (73) was converted to the corresponding thiolsulfinate by treatment with m-chloroperbenzoic acid. This was then reacted with hexaethylphosphorus triamide and N-t-butyloxycarbonyl-L-cysteine t-butyl ester, to give unsymmetrical cystine 75 in 43% yield.



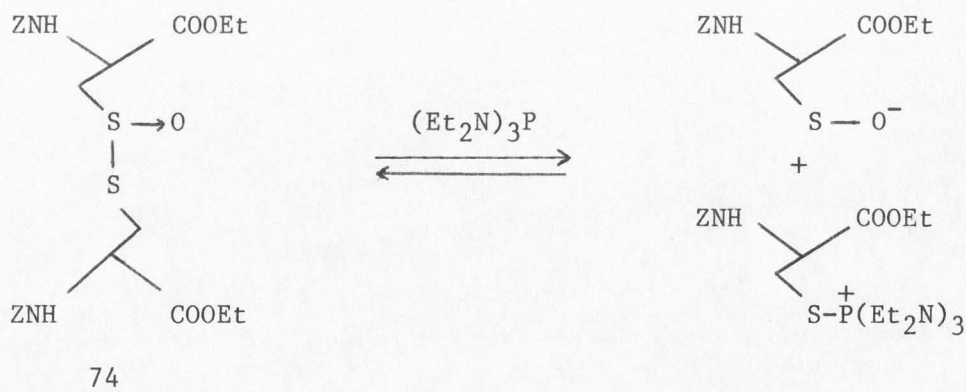
90 MHz ¹H NMR of 75 showed the absorption peaks for the four cystine methylenes as a triplet at δ 3.2 ppm. Apparently, this triplet results from two overlapping doublets, the two pairs of cystine methylenes being nonequivalent in the unsymmetrical cystine. The same reaction, when carried out under N₂, resulted in the formation of 75 in 55% yield. The N₂ atmosphere would minimize formation of cystine 72 by the oxidation of cysteine 70.

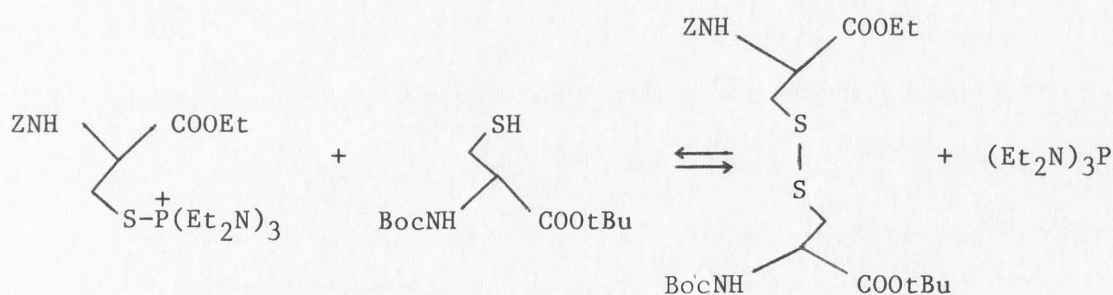
Treatment of unsymmetrical cystine 75 with hexaethylphosphorus triamide resulted in the formation of lanthionine 76 as the major product, in 52% isolated yield, with small amounts of symmetrical disulfides 72 and 73 as identified by tlc analysis and by NMR analysis.



^1H NMR of 76 showed the lanthionine methylenes resonating as a triplet centered at δ 3.0 ppm, as against δ 3.2 ppm in the case of cystine 75. Combustion analysis data were in agreement with calculated values, thus confirming the disulfide contraction. Hence, it was established that the contraction can, indeed, be effected on an unsymmetrical cystine.

Attempts to convert thiolsulfinates 74 directly to 76 by the use of an excess of hexaethylphosphorus triamide resulted in the formation of 75 in 15-22% yields. A possible explanation for these observations could be an equilibrium shown below.



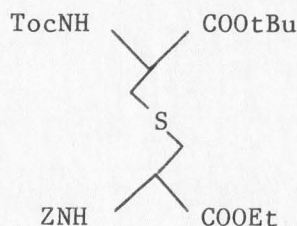


Thus an excess of hexaethylphosphorus triamide would shift the equilibrium to the left, which could then lead to other side reaction products.

Lanthionine 76 is completely unsymmetrical with four different protecting groups on the two amino and two carboxy terminals. These groups are compatible with each other and can be selectively removed in the proper sequence necessary in the proposed synthesis of 4. The ethyl ester can be hydrolyzed with aqueous base without hydrolysis of the t-butyl ester. The Boc group and the t-butyl ester are both susceptible to acidolytic cleavage. However, conditions for the selective removal of the Boc group in the presence of t-butyl ester have been reported.¹⁰⁴ The removal of both the Boc and t-butyl ester functions will not affect the depsipeptide ester bonds present, subsequently. The benzyloxycarbonyl group can be removed in the last step of the reaction sequence, before cyclization. Thus, in 76, the synthesis of an unsymmetrical lanthionine suitable for our proposed synthesis of bicyclic octapeptide lanthionine 4 has been accomplished.

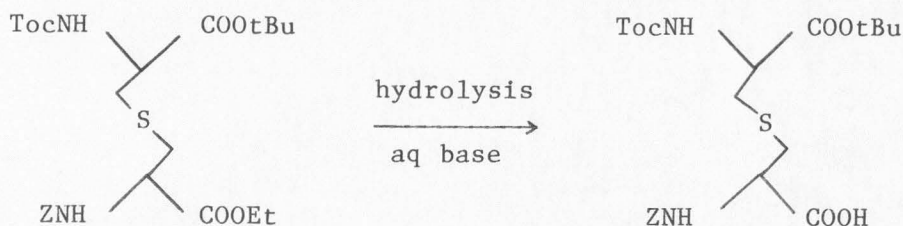
The method of synthesis of unsymmetrical lanthionine 76 via the corresponding thiol-sulfinate was applied to the synthesis of another unsymmetrical lanthionine 80. Lanthionine 80 has the 2,2,2-trichloroethyloxycarbonyl (Toc)¹⁰⁵ group on the amine nitrogen in lieu

of the Boc group present in 76. The advantage of this protecting group is that it can be removed by reductive cleavage with zinc in acetic acid. This aspect of removal of the Toc group facilitates the



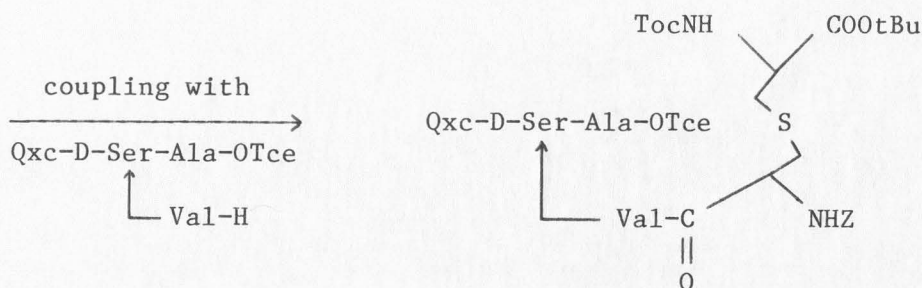
80

synthesis of octapeptide lanthionine 4, in that the trichloroethyl ester function is also cleaved under the same conditions, and hence both the amine and carboxyl group can be deprotected in one step. A schematic route proposed for the synthesis of lanthionine 4 is shown below.

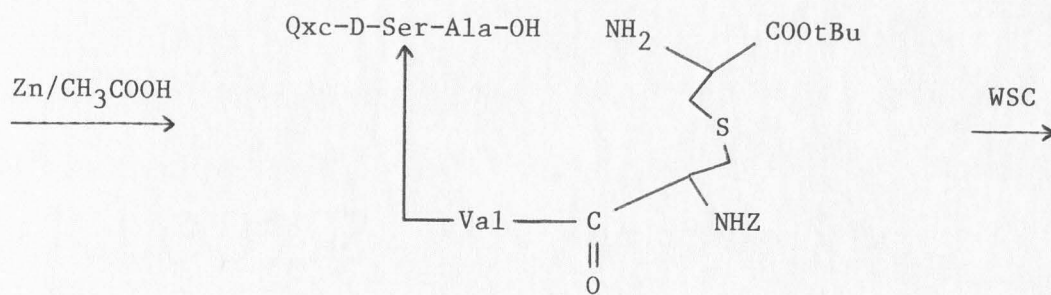
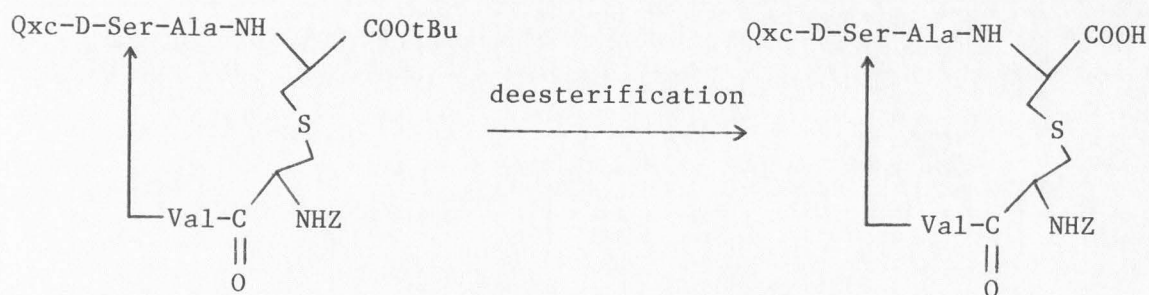
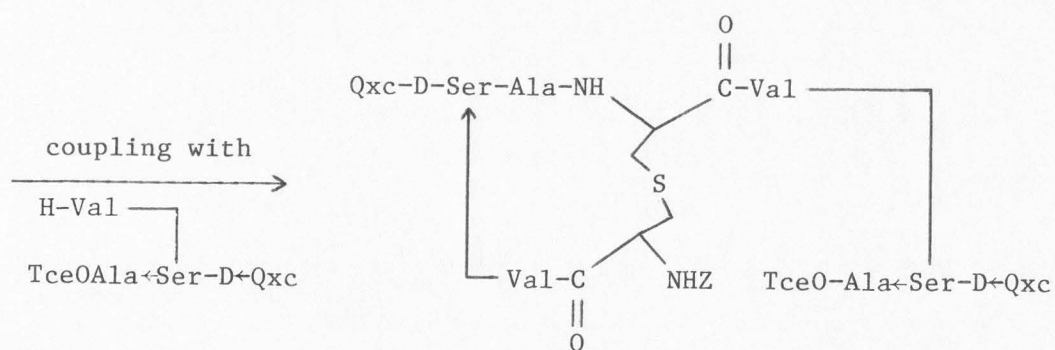


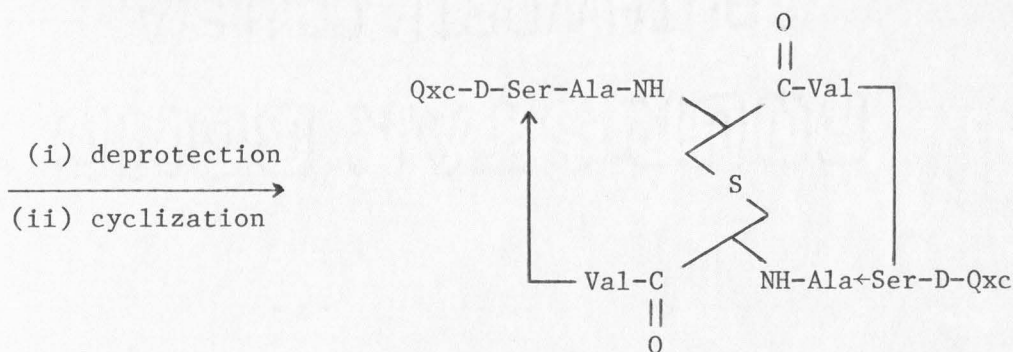
80

Toc: 2,2,2-trichloroethyloxycarbonyl



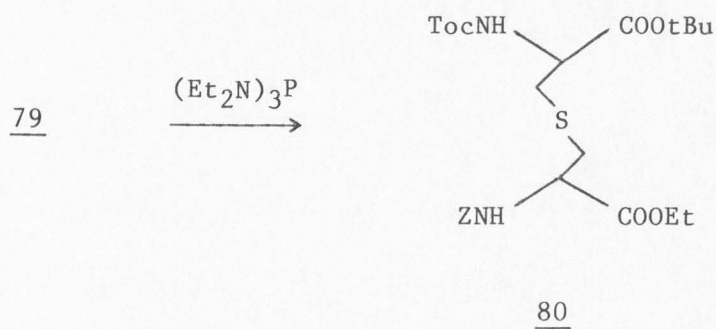
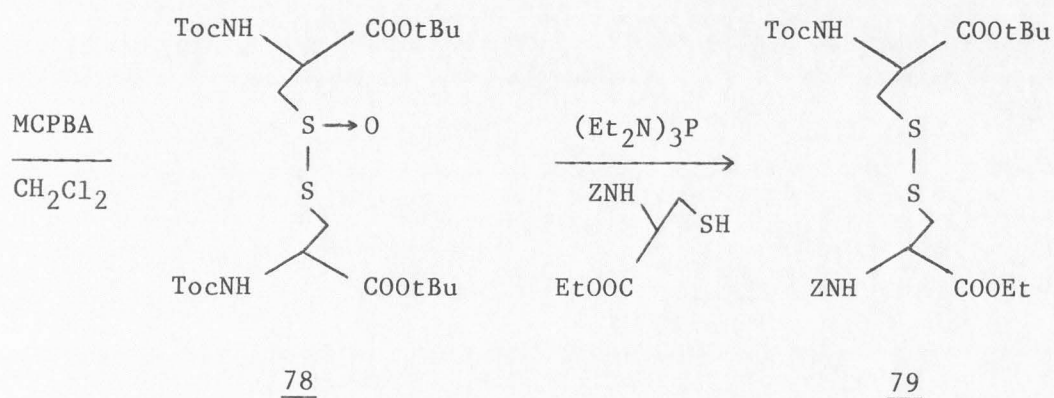
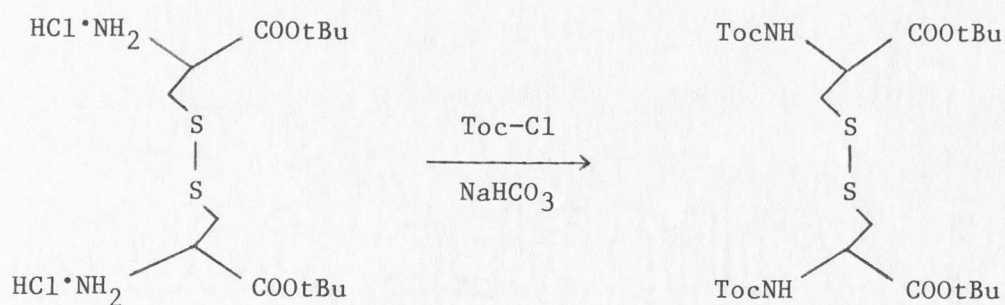
81

82838485

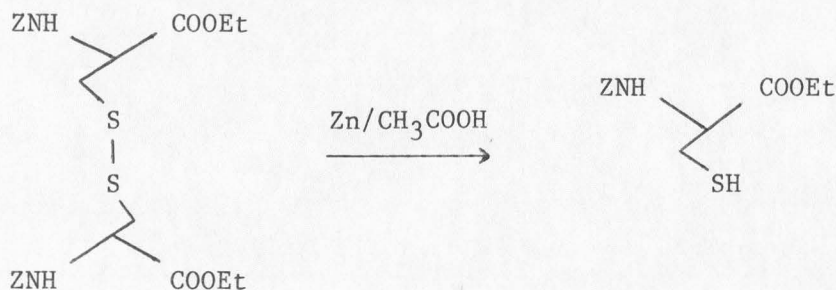


4

N,N'-Di-2,2,2-trichloroethoxycarbonyl-L-cystine t-butyl ester 77 was synthesized by treatment of L-cystine di-t-butyl ester dihydrochloride with 2,2,2-trichloroethoxycarbonyl chloride and NaHCO_3 in aqueous solution in 84% yield after purification on the mplc. 90 MHz ^1H NMR of 77 showed the absorption peak for the 2,2,2-trichloroethoxycarbonyl methylenes as a sharp singlet at δ 4.8 ppm. Elemental analysis data were in satisfactory agreement with the theoretical values. 77 was treated with m-chloroperbenzoic acid in CH_2Cl_2 resulting in the corresponding thiolsulfinate 78. The thiolsulfinate 78 was treated, without further purification, with one equivalent of hexaethylphosphorus triamide and N-benzyloxycarbonyl-L-cysteine ethyl ester in benzene solution under an atmosphere of nitrogen. The reaction mixture was purified on the mplc and unsymmetrical disulfide 79 was isolated in 55% yield. ^1H NMR of 79 showed the absorption of the cystine methylenes as a doublet at δ 3.2 ppm. The 2,2,2-trichloroethoxycarbonyl methylenes resonated as a singlet at δ 4.8 ppm. Combustion analysis data were in satisfactory agreement with calculated values.

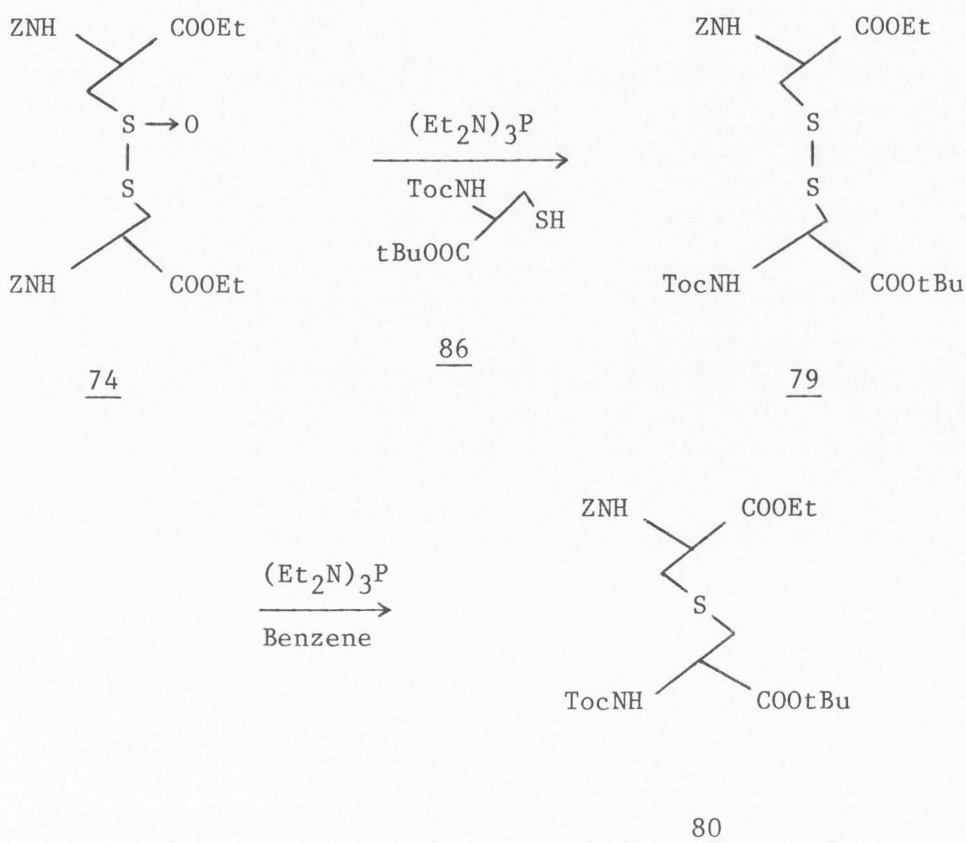


N-Benzylloxycarbonyl-L-cysteine ethyl ester was synthesized by reductive cleavage of the corresponding cystine with zinc in acetic acid.



79 was treated with 1.1 equivalents of hexaethylphosphorus triamide in benzene solution. The reaction product was purified on the mplc and the fraction having the same Rf value as for 79 was isolated. 90 MHz ^1H NMR of this fraction indicated it to be a mixture of lanthionine 80 and cystine 79. The cystine methylenes of 79 resonated as a doublet at δ 3.2 ppm while the lanthionine methylenes in 80 appeared as a doublet at δ 3.0 ppm. From the intensities of the peaks, 80 was found to be the major product. Treatment of 79 with 2.5 equivalents of hexaethylphosphorus triamide resulted in the formation of lanthionine 80 in 58% yield. In comparison, the lanthionine contraction of cystine 75 to lanthionine 76 was effected by 1.1 equivalents of hexaethylphosphorus triamide. Lanthionine 76 has the t-butyloxycarbonyl group on one of the two amine nitrogens while 80 has the 2,2,2-trichloroethyloxycarbonyl group, with the rest of the protecting groups in 76 and 80 being identical. Thus, the 2,2,2-trichloroethyloxycarbonyl group influences the lanthionine contraction. The role of the protecting group in this reaction, however, is not understood.

The synthesis of lanthionine 80 was carried out with a different thiolsulfinate 74 using the same sequence of reactions as for the above synthesis of 80, as outlined below.



The synthesis of N-trichloroethyloxycarbonyl-L-cysteine t-butyl ester 86 by reductive cleavage with zinc and acetic acid had a potential problem in that the N-protecting group itself was susceptible to removal under these conditions. However, the reduction of the disulfide bond is effected under milder conditions using 5% acetic acid, whereas the removal of the protecting group needs 90% acetic acid. Attempts to synthesize 86 by the reductive cleavage of cystine 77 with zinc in 5% acetic acid were unsuccessful. The method of Erickson and Khan,¹⁰⁶ using tri-n-butylphosphine in aqueous methanol, resulted in the conversion of 77 to 86 in near quantitative yields. The product of this reaction could be used directly without further purification. This method of synthesis of 80 had two advantages over

the first one. Unsymmetrical cystine 79 was obtained in 65% yield as compared to 55% by the previous method. The synthesis of N,N'-di-2,2,2-trichloroethyloxycarbonyl-L-cystine di-t-butyl ester 77, which is the precursor of thiol sulfinic acid 78 and cysteine 86, is tedious as compared to that of N,N'-di-benzyloxycarbonyl-L-cystine diethyl ester, precursor to thiol sulfinic acid 74. In the reaction of thiolsulfinic acid with cysteine, only one half of the cystine molecule corresponding to the thiolsulfinic acid is incorporated into the unsymmetrical cystine. Thus, the use of the more easily obtained thiolsulfinic acid 74 was found to be facile in the synthesis of 80.

CONCLUSION

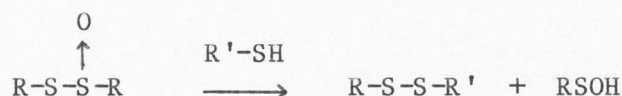
The attempted synthesis of bicyclic octadepsipeptide lanthionine 4 by the symmetrical approach was carried out. Symmetrical lanthionine N,N'-di-t-butyloxycarbonyl-L-lanthionine dimethyl ester (27) was prepared by a contraction of the corresponding cystine 26 using alkylaminophosphine, according to the method of Harpp and Gleason.⁶⁵ This method could not be applied to the lanthionine contraction of hexapeptide 35, cyclic octapeptide 13 and TANDEM. Apparently the reaction is sensitive to steric and conformational effects. Starting with 27, linear octapeptides 29 and 32 were prepared. Attempts to cyclize 29 and 32 to the bicyclic octapeptide, however, were unsuccessful.

The failure of the cyclizations of 29 and 32 led to the logical alternative, a suitably protected unsymmetrical lanthionine with four different protecting groups. These protecting groups have to be compatible within themselves as well as with the depsipeptide ester bonds present subsequently in the peptide intermediates.

Attempts to synthesize an unsymmetrical lanthionine by the nucleophilic displacement of the chloride ion from N-benzyloxycarbonyl- β -chloro-L-alanine with the thiol function of cysteine under phase transfer conditions, were unsuccessful. The displacement of the sulfonate-activated serine hydroxyl in L-serine containing peptides with mercaptide anion was studied. The displacement could not be effected when the C-terminus of the tosylated serine was incorporated as an amide bond with valine, starting tosylate being recovered in the

reaction. Valine as the C-terminal amino acid of the tosylated serine, appears to sterically hinder the nucleophilic displacement by mercaptide anion.

Unsymmetrical cystines were synthesized by the reaction of thiosulfinate with cysteine. To the best of our knowledge, this is the first example of the use of thiol sulfinates for the synthesis of a cystine or cystine peptide.



Suitably protected cystines were converted to the corresponding thiol sulfinates by peracid oxidation. Upon reaction with N- and C-protected cysteines, these led to the respective unsymmetrical cystines 75 and 76. Lanthionine contraction of these unsymmetrical cystines was effected with hexaethylphosphorus triamide in benzene solution. This is contrary to the observation of Harpp and Gleason,⁶⁵ who found this method unsuitable for the synthesis of unsymmetrical lanthionines.

The synthesis of two unsymmetrical lanthionines 76 and 80 with the appropriate protecting groups necessary for our proposed synthesis of octapeptide lanthionine 4 was accomplished. Further studies towards 4, however, were not pursued.

EXPERIMENTAL

Meltings points were determined with a Thomas-Hoover melting point apparatus, and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-360, XL-100-12, JEOL FX-90 or a Nicolet NT-360 spectrometer; satisfactory NMR data were obtained for all compounds and data for selected intermediates are reported. The format of the data report is: chemical shift (in δ units), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integral intensity, and source. Solvents were removed in vacuo on a Buchler rotary evaporator. Thin layer chromatography was performed on commercially available silica-gel plates; [Whatman MK6F-1 x 3", silica gel 40 $^{\circ}\text{A}$], with flourescent indicator; components were located under ultraviolet irradiation and with iodine vapors. The solvent systems used were: A) hexane-acetone, 8:2; B) hexane-acetone, 7:3; and C) hexane-acetone, 6:4. Medium pressure liquid chromatography (mplc) was performed on columns packed with silica gel 60 (0.040-0.064 mm).

The amino acids, their derivatives, coupling reagents and other chemicals used were obtained from commercial sources. The THF used was distilled from sodium benzophenone ketyl. DMF was distilled over CaH_2 and stored over appropriate molecular sieves. CH_2Cl_2 was distilled over P_2O_5 . All other solvents used were distilled in glass prior to use.

Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona.

Synthesis of N,N'-di-tert-butyloxy-carbonyl-L-cystine dimethyl ester (26)

To a stirred suspension of L-cystine dimethyl ester dihydrochloride (8.52 g, 25 mmol) in 125 ml of DMF, triethylamine (7.01 ml, 50 mmol) was added with stirring, followed by di-t-butyl dicarbonate (10.9 g, 50 mmol). After stirring at room temperature for 5 hr, the solvent was removed in vacuo and the residue was taken up in 10% citric acid. The aqueous phase was extracted four times with ethyl acetate; the combined organic phase was washed with sat NaHCO_3 , brine and dried over Na_2SO_4 . Upon removal of solvent in vacuo a white solid was obtained. This solid was crystallized from absolute ethanol-pet. ether to give 9.5 g (80%) of 26; m.p. 98-100°; reported m.p. 96-97°;¹⁰⁷ tlc (solvent A) Rf (0.23); ^1H NMR (60 MHz, CDCl_3) δ 1.5 (s, 18 H, Boc Me's), 3.2 (d, 4 H, cystine methylenes), 3.9 (s, 6H, methyl ester) 4.6 (m, 2H, α -H's), 5.8 (d, 2H, NH).

Synthesis of N,N'-di-tert-butyloxy-carbonyl-L-lanthionine dimethyl ester (27)

To a solution of 5 (8.53 g, 25 mmol) in benzene (80 ml) was added hexaethylphosphorus triamide (6.18 g, 25 mmol) dropwise, with stirring. The reaction mixture was stirred at room temperature for 1 hr. The solvent was removed in vacuo and the resulting oil purified on the medium pressure liquid chromatograph using a 25 mm diameter column; with 20% acetone in hexane as the eluting solvent system. Pure lanthionine 27 6.32 g (81%) was obtained as an oil, which solidified upon standing for several days; m.p. 69-72°; tlc (solvent A) Rf (0.23); ^1H NMR (60 MHz, CDCl_3) δ 1.5 (s, 18 H, Boc Me's), 3.0 (d, 4 H, lanthionine methylenes), 3.9 (s, 6H, methyl ester), 4.6 (m, 2H, α -H's),

5.8 (d, 2 H, NH). (Found: C, 49.17; H, 7.54; S, 7.8. $C_{18}H_{32}N_2O_8S$ requires: C, 49.54; H, 7.34; S, 7.34 %).

Synthesis of hexapeptide
lanthionine 28

A. Synthesis of N-benzyloxycarbonyl-O-(N-t-butyloxycarbonyl-L-valyl)-d-serine p-bromophenacyl ester. To N-benzyloxycarbonyl-D-serine p-bromophenacyl ester¹⁰⁸ (4.36 g, 10 mmol) in 60 ml pyridine was added N-t-butyloxycarbonyl-L-valine (3.24 g, 15 mmol), and the solution was cooled to 0° in an ice-salt bath. N,N'-dicyclohexylcarbodiimide (3.15 g, 15 mmol) was added, and the reaction mixture was stirred at 0° for 3-4 hr followed by stirring at room temp. overnight. The mixture was filtered, and the filtrate was concentrated on the vacuum evaporator to yield an oil. This oil was dissolved in ethyl acetate and washed with H₂O (50 ml), sat NaHCO₃ aq (2 x 25 ml), 10% citric acid (2 x 25 ml), and H₂O (50 ml). The organic phase was dried over Na₂SO₄ and concentrated to yield an oil. The oil was purified on the mpls with 20% acetone in hexane as elutant, to yield 5.4 g (85%) of a white solid; m.p. 95-96°; reported m.p. 97-98°. ⁴⁵ This compound was identical to an authentic sample.

B. Synthesis of 28. To a stirred solution of 27 (1.5 g, 3.4 mmol) in 80% aqueous dioxane was added to 2N NaOH dropwise to pH 10. The solution was maintained at pH 10 by subsequent addition of 2 N NaOH with stirring at room temp for 1/2 hr. The reaction mixture was washed with CH₂Cl₂ (20 ml) to remove unreacted diester, acidified in cold to pH 2 with 1 N HCl, and extracted with ethyl acetate (3 x 25 ml). The combined organic phase was washed with water, brine and dried over Na₂SO₄. Removal of solvent in vacuo yielded 1.17 g (86%) of diacid.

To the dipeptide synthesized in Step A (2.0 g, 3.15 mmol), was added 10 ml anhyd trifluoroacetic acid and the reaction mixture was stirred at room temp. for 1 hr. The solvent was removed in vacuo and the residue partitioned between sat NaHCO_3 aq and ethyl acetate. After separation, the organic phase was washed with sat NaHCO_3 aq (2 x 25 ml), H_2O (2 x 25 ml), and brine (50 ml). The solvent was dried over Na_2SO_4 and evaporated to yield an oil. To this oil, dissolved in THF (10 ml), was added a solution of the above diacid (0.61 g, 1 mmol) in THF (10 ml), and the mixture was cooled to 0° in an ice-salt bath. After addition of 1-hydroxybenzotriazole hydrate (0.93 g, 6.85 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.61 g, 3.2 mmol), the mixture was stirred at 0° for 1.5 hr, followed by stirring at room temp. overnight.

The solvent was removed in vacuo, and the residual oil was dissolved in CHCl_3 (50 ml). The organic phase was washed with water (50 ml), 1 N HCl (2 x 50 ml), sat NaHCO_3 aq (75 ml), water (25 ml) and brine in succession and dried over Na_2SO_4 . The solvent was removed in vacuo to yield a foam. Purification on the mpls with 30% acetone in hexane as eluting solvent led to 1.29 g (60%) of 28; tlc (solvent B) Rf: 0.2; ^1H NMR (360 MHz, CDCl_3) δ 0.9 (d, 12 H, Val Me's), 1.4 (s, 18 H, Boc Me's), 2.2 (m, 2 H, Val α -H's), 2.9 (d, 4 H, lanthionine methylenes), 4-6.5 (24 H, Ser methylenes, Ser and lanthionine α -H's, benzyloxycarbonyl and phenacyl methylenes, NH's), 7.4 (d, 10 H, benzyloxycarbonyl aromatic), 7.7 (A_2B_2 q, 8 H, phenacyl aromatic). (Found: C, 53.30; H, 5.58; N, 5.50; S, 2.31. $\text{C}_{64}\text{H}_{78}\text{O}_7\text{N}_6\text{Br}_2\text{S}$ requires: C, 53.26; H, 5.41; N, 5.80; S, 2.20 %).

Synthesis of octapeptide
lanthionine 29

To 28 (1.15 g, 0.8 mmol) was added trifluoroacetic acid (5 ml) and the mixture stirred at room temp. for 1 hr. After removal of solvent in vacuo, diethyl ether (5 ml) was added and the mixture concentrated to dryness, twice. To the residue, dissolved in 10 ml of THF, was added N-t-butyloxycarbonyl-L-alanine (0.32 g, 1.7 mmol), N-methylmorpholine (0.19 ml) and the reaction mixture cooled to 0°C in an ice-salt bath. 1-Hydroxybenzotriazole hydrate (0.22 g, 1.6 mmol) was added, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and the reaction mixture stirred at 0° for 3 hr followed by stirring overnight at room temperature.

The solvent was removed in vacuo and the residue dissolved in ethyl acetate (25 ml). The ethyl acetate extract was washed with water (25 ml), sat NaHCO₃ aq (15 ml), 1 N HCl (25 ml), sat NaHCO₃ (15 ml) and brine in succession. The ethyl acetate phase was dried over Na₂SO₄ and concentrated to an oil. The oil was purified on the mpls with 40% acetone in hexane as the elutant to yield 0.71 g (55%) of 29; tlc (solvent C) Rf 0.21; ¹H NMR (360 MHz, CDCl₃) δ 0.9 (d, 12 H, Val Me's), 1.3 (bs, 6 H, Ala Me's), 1.5 (s, 18 H, Boc Me's), 2.2 (m, 2 H, Val α-H's), 2.9 (bd, 4 H, lanthionine methylenes) 4-6.5 (24 H, Ser methylenes, Ser and Ala α -H's, benzyloxycarbonyl and phenacyl methylenes, NH), 7.4 (s, 10 H, benzyloxycarbonyl aromatic), 7.7 (A₂B₂ q, 8H, phenacylaromatic). (Found: C, 52.72; H, 5.50; N, 7.03. C₇₀H₈₈N₈O₂₂Br₂S requires: C, 53.03; H, 5.55; N, 7.07 %).

Attempted synthesis of octadepsipeptide
31 from 29

A stirred solution of 29 (0.61 g, 0.4 mmol) in 90% aqueous acetic acid (10 ml) was cooled to 0° in an ice-salt bath. Zinc powder (1.25 g, 19.2 mmol) was added slowly, in portions, over a period of 15 min and the reaction mixture stirred at 0° for 1.5 hr. The reaction mixture was filtered and the ppt washed well with 90% acetic acid. The filtrate and washings were combined and concentrated to dryness. The residue was partitioned between 1 N HCl (50 ml) and ethyl acetate-methanol (4:1 v/v, 50 ml). The organic phase was separated and the aqueous phase saturated with NaCl. The saturated aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with water twice, dried over Na₂SO₄, and concentrated to an oil.

To the above oil was added trifluoroacetic acid (5 ml) and the mixture was stirred at room temp for 1 hr. The mixture was evaporated to dryness in vacuo, diethyl ether (2 x 5 ml) was added and concentrated to dryness. The residue was dissolved in anhyd N,N-dimethylformamide (30 ml) and cooled to 0° in an ice-salt bath. N-Methylmorpholine (0.09 ml, 0.8 mmol) was added followed by N-hydroxysuccinimide (0.19 g), and the reaction mixture diluted with THF (500 ml). After cooling to 0°, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was added and the mixture stirred at 0° for 3 hr and at room temp. for 5 days.

Handwritten:
Mmoles
of EDC?

The reaction mixture was concentrated in vacuo and residue dissolved in ethyl acetate (75 ml). The ethyl acetate extract was then filtered to remove solid and washed ppt washed well with ethyl acetate. The combined organic phase was washed with 1 N HCl (25 ml), sat NaHCO₃

aq (25 ml) and water (50 ml) in succession, dried over Na_2SO_4 and concentrated to an oil.

Tlc analysis (solvents B and C) showed the product to be a complex mixture, with no trace of desired cyclic octapeptide 31. An authentic sample with a disulfide linkage, a known intermediate in the synthesis of TANDEM, was used for comparison.

Repeating the above procedure with CH_2Cl_2 in lieu of THF as solvent gave similar results.

Synthesis of octapeptide 32

To N-benzyloxycarbonyl-O-(t-butyloxycarbonyl-L-valyl)-D-seryl-L-alanine 2,2,2-trichloroethyl ester²¹ (3.84 g, 6 mmol) was added trifluoroacetic acid (10 ml), CH_2Cl_2 (10 ml) and the mixture was stirred at room temp. for 1 hr. The solvent was removed in vacuo, anhyd ether (2 x 10 ml) added, and evaporated to dryness. To the residue was added anhyd ether again (50 ml) and the flask scratched with a spatula. Upon cooling in the refrigerator for 2 hours, a white crystalline ppt was obtained. The solid was separated by filtration, washed well with anhyd ether and dried in the dessicator overnight.

To the dried trifluoroacetate salt was added CH_2Cl_2 (10 ml) and N-methylmorpholine (1.2 ml), when the solution went clear. To this clear solution was added a solution of N,N'-di-tertbutyloxycarbonyl-L-lanthionine (diacid obtained upon hydrolysis of 27) (1.23 g, 3 mmol) in CH_2Cl_2 (65 ml). The mixture was cooled to 0° in an ice-salt bath. After addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.2 g, 6.3 mmol) and 1-hydroxybenzotriazole monohydrate (0.14 g, 0.9 mmol), the reaction mixture was stirred at 0° for 4 hr followed by stirring at room temp. overnight.

Solvent was removed in vacuo and the residue dissolved in ethyl acetate (75 ml). The organic phase was washed with H₂O (50 ml), sat NaHCO₃ aq (50 ml), 10% citric acid (50 ml), sat NaHCO₃ aq (50 ml) and H₂O (2 x 25 ml) in succession, and dried over Na₂SO₄. Upon evaporation of solvent, an oil was obtained. The oil was purified on the mplc with 30% acetone in hexane as the elutant, to yield 2.5 g (57.3%) of 32 as an oil; tlc (solvent B) Rf: 0.22; ¹H NMR (360 MHz, CDCl₃) δ 0.9 (d, 12 H, Val Me's), 1.3-1.5 (overlapping s, 24 H, Boc, Ala Me's), 2.2 (m, 2 H, Val α-H's), 2.9 (bd, 4 H, lanthionine methylenes) 4-6.5 (24 H, Ser methylenes, Ser and Ala α-H's, benzyloxycarbonyl and trichloroethyl methylenes, NH), 7.2 (s, 10 H, benzyloxycarbonyl aromatics). (Found: C, 48.0; H, 5.69; N, 7.57. C₅₈H₈₀Cl₆N₈O₂₀S requires: C, 47.9; H, 5.51; N, 7.71 %).

Attempted synthesis of cyclic octapeptide 31 from 32

To a stirred cold solution of 32 (2.18 g, 1.5 mmol) in 90% aqueous acetic acid (35 ml), was added zinc powder (4.7 g, 72 mmol) slowly, in portions, over a period of 20 min with stirring. The mixture was subsequently stirred at 0° for 1.5 hr. The reaction mixture was filtered and the ppt washed well with 90% aqueous acetic acid. The filtrate and washings were combined and concentrated in vacuo to dryness. The residue was partitioned between 1 N HCl (75 ml) and ethyl acetate-methanol (4:1 v/v, 100 ml). The organic phase was separated and the aqueous phase saturated with NaCl. The saturated aqueous phase was extracted with ethyl acetate (2 x 50 ml). The combined ethyl acetate extract was washed several times with H₂O, and dried over Na₂SO₄. Evaporation of solvent gave 1.6 g of an oil.

To this oil was added trifluoroacetic acid (10 ml) and the solution stirred at room temp. for 1 hr, then concentrated to a sticky residue. To the residue was added ether (10 ml), triturated well and evaporated to complete dryness using a high vacuum rotavapor.

mmoles?
The residue was dissolved in 10 ml anhyd dimethylformamide and cooled to 0° in an ice-salt bath. N-Hydroxysuccinimide and N-methylmorpholine were added, and the reaction mixture diluted with 500 ml THF. After cooling to 0°, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was added and the mixture stirred at 0° for 3 hr and at room temp. for 5 days.

The reaction mixture was concentrated in vacuo and the residue dissolved in ethyl acetate (100 ml). Insoluble material was removed by filtration and the ppt washed well with ethyl acetate. The combined washings and filtrate were washed with 1 N HCl (2 x 50 ml), sat NaHCO₃ aq (50 ml) and H₂O (50 ml).

TLC analysis of product (solvents B and C) showed the product to be a complex mixture, with no desired octapeptide 31.

Synthesis of tetrapeptide 34

N,N'-Di-t-butyloxycarbonyl-L-cystine (0.88 g, 2 mmol) and L-valine methyl ester (0.52 g, 4 mmol) were dissolved in pyridine (10 ml) and the solution cooled to 0° in ice-salt bath. N,N'-dicyclohexylcarbodiimide (0.88 g, 4.2 mmol) was added and the reaction mixture stirred at 0° for 3 hr and room temperature overnight.

The reaction mixture was filtered and ppt washed with pyridine. The combined filtrate and washings were evaporated in vacuo and the residue dissolved in ethyl acetate (25 ml). The organic phase was washed with sat NaHCO₃ aq (2 x 25 ml) and dried over MgSO₄. Solvent

was removed in vacuo to yield a white solid which crystallized from hexane-acetone to give 1g (75%) of 45; m.p. 81-82°; tlc (solvent-C) Rf 0.42; ^1H NMR (60 MHz, CDCl_3) δ 1.0 (d, 12 H, Val Me's), 1.5 (s, 18 H, Boc Me's), 3.2 (d, 4 H, cystine methylenes), 3.8 (s, 6 H, methyl ester), 4.7 (m, 2 H, α -H's).

Lanthionine contraction of tetrapeptide 34

To tetrapeptide 34 (1.33g, 2 mmol) in benzene (10 ml) was added hexaethylphosphorus triamide (0.6 g, 2.4 mmol) dropwise, with stirring, and the mixture was stirred at room temp. for 2 hr. Solvent was removed in vacuo to yield an oil. The oil was purified on the mpls with 20% acetone in hexane as the eluting solvent to give 0.4 g (31%) of lanthionine as an amorphous solid; tlc (solvent C) Rf 0.42; ^1H NMR (60 MHz, CDCl_3) δ 1.0 (d, 12 H, Val Me's), 1.5 (s, 18 H, Boc Me's), 3.0 (d, 4 H, lanthionine methylenes), 3.8 (s, 6H, methyl ester), 4.7 (m, 2 H, α -H's). (Found: C, 53.18; H, 8.02; N, 9.28. $\text{C}_{28}\text{H}_{50}\text{N}_4\text{O}_{10}\text{S}$ requires: C, 52.99; H, 7.89; N, 8.83%).

Synthesis of hexapeptide 35

To N-benzyloxycarbonyl-O-(N-t-butyloxycarbonyl-L-valyl)-D-serine p-bromophenacyl ester⁴⁵ (2.0 g, 3.2 mmol) was added trifluoroacetic acid (10 ml) and the mixture was stirred at room temp. for 1 hr. The mixture was concentrated on the rotavapor, and the residue dissolved in diethyl ether (100 ml). The organic phase was washed with sat NaHCO_3 aq (2 x 50 ml) and H_2O (50 ml), and dried over MgSO_4 . Removal of the solvent led to an oil. To a stirred solution of this oil in THF (20 ml) was added N,N'-di-t-butyloxycarbonyl-L-cystine (0.66 g, 1.5 mmol)

and 1-hydroxybenzotriazole monohydrate (0.93 g, 6.9 mmol). The mixture cooled to 0° in ice-salt bath. To the chilled solution, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride was added, and the reaction mixture stirred at 0° for 2 hr, followed by stirring at room temp. overnight.

Solvent was removed in vacuo and the resulting oil dissolved in CHCl₃ (100 ml). The organic phase was washed with H₂O (50 ml), 2 N HCl (2 x 50 ml), sat NaHCO₃ aq (2 x 50 ml), H₂O (50 ml) and brine (25 ml) in succession and dried over MgSO₄. Removal of the solvent led to a foam which was purified on mplc with 30% acetone in hexane as the eluting solvent system, to yield 1.1 g (50%) of 35; tlc (solvent B) Rf: 0.2; ¹H NMR (60 MHz, CDCl₃) δ 0.9 (d, 12 H, Val Me's), 1.4 (s, 18 H, Boc Me's), 2.2 (m, 2 H, Val α-H's), 3.2 (d, 4 H, cystine methylenes), 4-6.5 (22 H, Ser methylenes, Ser and cystine α-H's, benzyloxycarbonyl and phenacyl methylenes, NH), 7.4 (s, 10 H, benzyloxycarbonyl aromatic), 7.7 (A₂B₂q, 8 H, phenacyl aromatics). (Found: C, 52.31; H, 5.26; N, 5.83. C₆₄H₇₈O₂₀N₆Br₂S₂ requires: C, 52.10; H, 5.29, N, 5.7%).

Attempted lanthionine contraction
of above hexapeptide 35

To hexapeptide 35 (1.1 g, 0.75 mmol) in benzene (10 ml) was added hexaethylphosphorus triamide (0.21 g, 0.83 mmol) and the mixture stirred at room temp. for 2.5 hr. The reaction mixture was concentrated in vacuo and the resulting oil purified on the mplc using 30% acetone in hexane as elutant. ¹H NMR spectrum of isolated product was identical to that of hexapeptide 35. Hence contraction had not occurred.

Synthesis of N-benzyloxycarbonyl-L-seryl-L-valine methyl ester

To L-valine methyl ester hydrochloride (0.84 g, 5.0 mmol) in CH_2Cl_2 (40 ml) was added triethylamine (0.7 ml, 5.0 mmol) and the mixture cooled to 0° in ice-salt bath. N-Benzyloxycarbonyl-L-serine (1.2 g, 5.0 mmol) was added followed by N,N'-dicyclohexylcarbodiimide (1.13 g, 5.0 mmol) and the reaction mixture was stirred at 0°C for 3 hr and at room temp. overnight.

The mixture was filtered and the ppt washed well with CH_2Cl_2 . Removal of solvent in vacuo led to a white solid, which was dissolved in ethyl acetate (50 ml), and the resulting solution filtered to remove solid that separated out. The filtrate was washed with sat NaHCO_3 aq (25 ml), 10% citric acid aq (25 ml), sat NaHCO_3 aq (25 ml), H_2O (25 ml) and brine in succession. The organic phase was dried over Na_2SO_4 and solvent removed in vacuo to yield a white solid which crystallized from ethyl acetate:ether to yield 1.25 g (71%) of dipeptide; m.p. $140-142^\circ$; tlc (solvent B) Rf: 0.2; ^1H NMR (60 MHz, CDCl_3) δ 0.9 (dd, Val Me's), 3.8 (s, 3 H, Me ester), 4.4 (m, 2 H, serine methylenes), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 6.1 (bd, 2 H, NH's), 7.5 (s, 5 H, benzyloxycarbonyl aromatics).

Synthesis of N-benzyloxycarbonyl-O-(p-toluenesulfonyl)-L-seryl-L-valine methyl ester (61)

To the above dipeptide, N-benzyloxycarbonyl-L-seryl-L-valine methyl ester (1.06 g, 3 mmol), dissolved in pyridine (10 ml) and cooled to 0° in ice-salt bath, was added p-toluenesulfonyl chloride (0.63 g, 3.3 mmol), followed by 4-dimethylamino pyridine (0.04 g, 0.3 mmol). The

mixture was kept at -10° for 1.5 hr in CCl_4 -dry ice bath and at room temp. for an additional hour.

The solvent was removed in vacuo and ice-water added to the residue. The aqueous mixture was extracted with ethyl acetate (50 ml), and the organic extract washed quickly with 5% HCl (25 ml) and brine in succession. Removal of solvent led to an oil which was purified by flash chromatography with 30% acetone in hexane as the eluting solvent system to yield 0.9 g (60%) of 61 as an oil; tlc (solvent B) Rf: 0.35; ^1H NMR (60 MHz, CDCl_3) δ 0.9 (dd, Val Me's), 2.0 (m, 1H, Val α -H), 2.5 (s, 3 H, p-toluenesulfonyl methyl), 3.8 (s, 3 H, Me ester) 4.4 (m, 4 H, serine methylenes, α -H's), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 7.2-8.0 (9 H, benzyloxycarbonyl, p-toluenesulfonyl aromatics).

Attempted displacement of 61
with benzyl mercaptan

To benzyl mercaptan (0.08 ml, 0.65 mmol) in dimethylformamide (5 ml) was added triethylamine (0.09 ml, 0.65 mmol) and the mixture stirred for 5 min. Tosylate 61 (0.33 g, 0.65 mmol) was added, and the mixture stirred at 50° for 3 hr and allowed to stand overnight at room temp.

Solvent was removed in vacuo and ice water (5 ml) was added to the residue. The aqueous mixture was extracted with ethyl acetate and the organic phase washed with 1 N HCl (5 ml), H_2O (5 ml) and brine in succession. The organic phase was dried over Na_2SO_4 and solvent removed in vacuo to yield an oil. The two major spots on tlc were separated by flash chromatography. One corresponded to starting tosylate 61. The NMR of the other product was consistent for dibenzyl disulfide. No displacement product was isolated.

Use of sodium methoxide in lieu of triethylamine as base gave identical results.

Displacement of N-benzyloxycarbonyl-
O-(p-toluenesulfonyl)-L-seryl-glycine
methyl ester with benzyl mercaptan

To benzyl mercaptan (0.14 ml, 1.22 mmol) in methanol (2.5 ml) was added sodium methoxide (0.07 g, 1.22 mmol) and the mixture was stirred for 5 min. Solvent was removed in vacuo and the residue was dissolved in dimethylformamide (5 ml). N-benzyloxycarbonyl-O-(p-toluenesulfonyl)-L-seryl-glycine methyl ester (0.15 g, 0.32 mmol) was added and the mixture stirred at room temp. for 1 hr.

Solvent was removed in vacuo, and to the residue was added ice-water (5 ml) and 0.1 N HCl (5 ml). The aqueous mixture was extracted with ethyl acetate (15 ml) and the organic phase washed with 0.1 N HCl (5 ml), H₂O (5 ml) and brine in succession, and dried over Na₂SO₄. Removal of solvent resulted in an oil which was purified on a gravity column with 20% acetone in hexane as the eluting solvent system to yield 0.08 g (60%) of N-benzyloxycarbonyl-S-benzyl-L-cysteinyl glycine methyl ester; tlc (solvent B) R_f 0.41; ¹H NMR (60 MHz, CDCl₃) δ 2.9 (d, 2 H, cysteine methylenes), 3.8 (s, 3 H, methyl ester), 4.1 (d, 2 H, Gly methylenes), 4.5 (m, 2 H, Ser methylenes), 5.2 (s, 2 H, benzyloxy-carbonyl methylenes), 5.8 (d, 1 H, NH), 7.0 (bs, 1 H, NH), 7.4 (d, 10 H, benzyloxycarbonyl, s-benzyl aromatic).

Synthesis of tripeptide 63

To dipeptide N-benzyloxycarbonyl-O-(N-t-butyloxycarbonyl-L-valyl)-D-serine p-bromophenacyl ester ⁴⁵ (0.95 g, 1.5 mmol) was added trifluoroacetic acid (5 ml) and the mixture stirred at room temp. for 1

hr. The reaction mixture was concentrated on the rotavapor and to the residue was added anhyd ether (5 ml). After trituration with ether, the solvent was removed in vacuo; this treatment with ether was carried out twice, and solvent removed in vacuo to yield a white solid.

To the white solid in CH_2Cl_2 (15 ml) was added triethylamine (0.21 ml, 1.65 mmol) followed by N-t-butyloxycarbonyl-L-serine (0.30 g, 1.5 mmol). The mixture was cooled to 0° in an ice-salt bath and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.29 g, 1.52 mmol) was added, followed by 1-hydroxybenzotriazole monohydrate (0.20 g, 1.5 mmol). The mixture was stirred at 0° for 4 hrs and at room temp. overnight.

The solvent was removed in vacuo and the residue dissolved in ethyl acetate (50 ml). The organic phase was washed with H_2O (25 ml), sat NaHCO_3 aq (25 ml), 1 N HCl (25 ml), sat NaHCO_3 aq (25 ml) and sat NaCl aq (15 ml) in succession. Drying the organic phase over Na_2SO_4 and evaporation of solvent led to 1.06 g (98%) of 63 as a foam; tlc (solvent B) R_f 0.2, ^1H NMR (60 MHz, CDCl_3) δ 1.0 (dd, 6 H, Val Me's), 1.5 (s, 9 H, Boc Me's), 2.2 (m, 1 H, Val α -H), 4-5 (6 H, Ser methylenes, α -H's), 5.2 (s, benzyloxycarbonyl methylenes), 5.4 (s, 2 H, p-bromophenacyl methylenes) 7.5 (s, 5 H, benzyloxycarbonyl aromatics), 7.8 (q, 4 H, p-bromophenacyl aromatics).

Synthesis of tripeptide mesylate 65

To the tripeptide 63 (0.2 g, 0.28 mmol) in pyridine (2 ml), cooled to 0° in ice-salt bath, was added methanesulfonyl chloride (0.024 ml, 0.31 mmol). The mixture was stirred at 0° for 1 hr and cooled in the refrigerator overnight.

Solvent was removed in vacuo, and the residue dissolved in ethyl acetate (10 ml). The organic phase was washed with cold 1 N HCl (5 ml), H₂O (5 ml) and dried over Na₂SO₄. Removal of solvent led to 0.22 g of 65 (96%) as an oil; tlc (solvent B) R_f 0.23, ¹H NMR (60 MHz, CDCl₃) δ 0.9 (dd, 6 H, Val Me's), 1.5 (s, 9 H, Boc Me's), 3.0 (s, 3 H, methyl), 4-5 (6 H, Ser methylenes α-H's), 5.2 (s, 2 H, benzyloxycarbonyl aromatics), 7.8 (A₂B₂ q, 4 H, p-bromophenacyl aromatics).

Attempted displacement of 65 with benzyl mercaptan

To benzyl mercaptan (0.06 ml, 0.6 mmol) in methanol (2 ml) was added sodium methoxide (0.03 g, 0.55 mmol) and the mixture stirred for 5 min. To this mixture was added 65 (0.4 g, 0.5 mmol) in dimethylformamide (5 ml) and the mixture stirred at room temp. for 1.5 hr.

Solvent was removed in vacuo and ice water (10 ml) added to the residue. The aqueous phase was extracted with ethyl acetate (10 ml), and the organic phase washed with 1 N HCl (5 ml), H₂O and brine in succession. The organic phase was dried over Na₂SO₄ and solvent removed in vacuo to yield an oil. The NMR spectrum of the oil indicated a mixture of 65 and benzyl mercaptan. Tlc showed the major spots corresponding to 65 and benzyl mercaptan.

Synthesis of N-benzyloxycarbonyl-L-serine-(N-t-butyloxycarbonyl)-hydrazide (66)

To N-benzyloxycarbonyl-L-serine (1.2 g, 5 mmol) in CH₂Cl₂ (40 ml) was added N-t-butyloxycarbonyl hydrazine (0.73 g, 5.5 mmol) and the mixture cooled to 0° in ice-salt bath. N,N'-Dicyclohexycarbodiimide

was added, and the mixture stirred at 0° for 3 hr followed by stirring at room temp. overnight.

The reaction mixture was filtered and the ppt washed with CH₂Cl₂. The combined filtrate and washings were concentrated in vacuo and the residue dissolved in ethyl acetate (50 ml), when small amounts of solid separated. The ethyl acetate extract was filtered, and the filtrate washed with sat NaHCO₃ aq (25 ml), 1 N HCl (25 ml), sat NaHCO₃ aq (25 ml) and sat NaCl aq (15 ml) in succession. Drying of the organic phase over Na₂SO₄ followed by evaporation of solvent in vacuo resulted in an oil. The oil was purified on a silica gel gravity column, to yield 1.68 g (100%) of (66); tlc (solvent B) Rf 0.34, ¹H NMR (60 MHz, CDCl₃) δ 1.5 (s, 9 H, Boc Me's), 3.7-4.6 (3 H, Ser methylenes, α-H), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 6.2 (d, 1 H, NH), 7.1 (s, 1 H, NH), 7.4 (s, 5 H, benzyloxycarbonyl aromatics), 8.8 (s, 1 H, NH).

Synthesis of N-Benzyloxycarbonyl-
O-(p-toluenesulfonyl)-L-serine (N-
t-butyloxycarbonyl) hydrazide (67)

To 66 (0.54 g, 1.6 mmol) in pyridine (10 ml), cooled to 0° in ice-salt bath was added p-toluenesulfonyl chloride (0.32 g, 1.68 mmol) and the mixture stirred at 0° for 1 hr, followed by standing overnight in the refrigerator.

The reaction mixture was concentrated in vacuo and ice-water (10 ml) added to the residue. The aqueous residue was extracted with ethyl acetate (25 ml) and the ethyl acetate extract washed quickly with cold 1 N HCl (15 ml). The organic phase was subsequently washed with H₂O (10 ml) and brine in succession. After drying the organic phase over Na₂SO₄, solvent was removed in vacuo to yield 0.77 g (97.5 %) of pure 67 as an oil; tlc (solvent B) Rf 0.51, ¹H NMR (60 MHz, CDCl₃) δ 1.5

(s, 9 H, Boc Me's), 2.5 (s, 3 H, p-toluenesulfonyl methyl), 4.0-4.9 (3 H, Ser methylenes, α -H), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 6.2 (d, 1 H, NH), 6.9 (s, 1 H, NH), 7-8 (q overlapping s, 9 H, benzyloxycarbonyl and p-toluenesulfonyl aromatics), 8.8 (s, 1 H, NH).

Attempted displacement of 67
with benzyl mercaptan

To benzyl mercaptan (1.04 ml, 1 mmol) in methanol (2 ml) was added a solution of sodium methoxide (0.06 g, 1.1 mmol) in methanol (2 ml) and the mixture stirred for 5 min. 0.51 g (1 mm) of 67 in dimethylformamide (5 ml) was added and the mixture stirred at room temp. for 2 hr.

Solvent was removed in vacuo and ice water (5 ml) was added to the residue. The aqueous mixture was extracted with ethyl acetate (10 ml). The ethyl acetate phase was washed with 1 N HCl (5 ml), H₂O and brine in succession. Upon drying over Na₂SO₄ and removal of solvent, an oil was obtained. The NMR of this oil was consistent for the product, a cyclic intramolecular displacement of the tosylate (see discussion). ¹H NMR (60 MHz, CDCl₃) 1.5 (s, 9 H, Boc Me's), 3.8 (bd, 2 H, methylenes), 5.2 (s, 2 H, benzyloxycarbonyl methylenes) 6.2 (bd, NH), 7.4 (s, 5 H, benzyloxycarbonyl aromatic), 9.2 (s, 1 H, NH).

Synthesis of N,N'-di-t-butyloxy-
carbonyl-L-cystine di-t-butyl
ester (72)

To a suspension of L-cystine di-t-butyl ester dihydrochloride¹⁰⁰ (1.28 g, 3 mmol) in dimethylformamide (30 ml) was added triethylamine (0.84 ml, 6 mmol) and the mixture stirred for 5 min. Di-t-butyl

dicarbonate (1.46 g, 6.6 mmol) was added and the reaction mixture stirred at room temp. for 5 hr.

Solvent was removed in vacuo and the residue partitioned between 1 N HCl (50 ml) and ethyl acetate (75 ml). The organic phase was washed with 1 N HCl (2 x 25 ml), H₂O (25 ml) and brine in succession, and dried over Na₂SO₄. Removal of solvent led to an oil which was purified on mplc with 10% acetone in hexane as the eluting solvent to yield 1.52 g (91%) of 72 as a white solid; m.p. 97-99°; tlc (solvent B) R_f 0.69; ¹H NMR (60 MHz, CDCl₃) δ 1.5 (s, 36 H, Boc and t-butyl Me's), 3.2 (d, 4 H, cystine methylenes), 4.5 (m, 2 H, α-H's), 5.5 (bd, 2 H, NH).

Synthesis of N-t-butyloxycarbonyl-L-cysteine t-butyl ester (70)

To 72 (2.5 g, 4.65 mmol) in 5% acetic acid in ether (65 ml) cooled to 0° in ice-salt bath was added zinc dust (18.34 g, 282 mmol) slowly in portions over a period of 20 min. The mixture was stirred at 0° for 2 hr.

The mixture was filtered and the ppt washed well with acetic acid. The combined filtrate and washing was concentrated in vacuo and the residue taken up in 1 N HCl (75 ml). The aqueous phase was extracted with ethyl acetate (75 ml) and the organic phase washed with sat NaCl aq (50 ml). Upon drying over Na₂SO₄ and removal of solvent, 2.51 g (100%) of 70 was obtained as an oil; tlc (solvent CHCl₃) R_f 0.7; ¹H NMR (60 MHz, CDCl₃) δ 1.5 (s, 18 H, Boc and t-butyl Me's), 1.9 (d, 1 H, SH), 3.0 (dd, 2 H, cysteine methylenes), 4.5 (m, 1 H, α-H), 5.5 (bd, 1 H, NH). The oil was used in subsequent reactions without further purification.

N,N'-Di-benzyloxycarbonyl-L-cystine
diethyl ester (73)

To L-cystine diethyl ester dihydrochloride (7.38 g, 20 mmol) dissolved in H₂O (400 ml) and cooled to 0° in ice-salt bath, was added NaHCO₃ (6.72 g, 80 mmol) slowly, with stirring. To the cold solution was added benzyl chloroformate (8.6 ml, 60 mmol) dropwise and the reaction mixture stirred at 0° for 1 hr followed by stirring at room temp. for 5 hr.

The reaction mixture was saturated with NaCl and extracted with ethyl acetate (4 x 100 ml). The combined organic phase was washed with H₂O (100 ml) and dried over Na₂SO₄. Removal of solvent in vacuo resulted in a white solid which was crystallized from ethyl acetate-pet. ether to yield 9.8 g (88%) of 73; m.p. 81-83°; reported m.p. 86°;¹⁰⁹ tlc (solvent CHCl₃) R_f 0.21; ¹H NMR (60 MHz, CDCl₃) δ 1.3 (t, 6 H, Me's of ethyl ester), 3.2 (d, 4 H, cystine methylenes), 4.3 (q, 4H, methylenes of ethyl ester), 4.8 (m, 2H, α-H's), 5.2 (s, 4 H, benzyloxycarbonyl methylenes), 5.8 (d, 2 H, NH), 7.5 (s, 10 H, benzyloxycarbonyl aromatics).

Synthesis of thiol sulfinic acid 74

To N,N'-di-benzyloxycarbonyl-L-cystine diethyl ester (5.07 g, 9 mmol) in CH₂Cl₂ (150 ml) cooled to 0° in ice-salt bath, was added m-chloroperbenzoic acid slowly with stirring, when the solution turned turbid. The reaction mixture was allowed to warm up to room temp. upon which a clear solution resulted. The clear solution was allowed to stand overnight at room temp.

The mixture was washed well with sat NaHCO₃ aq (3 x 50 ml), sat NaCl aq (50 ml) and dried over anhyd Na₂SO₄. Removal of solvent

resulted in 5.2 g (100%) of 74 as an oil, which was used for subsequent reaction without purification; tlc (solvent B) Rf 0.15.

Synthesis of N-benzyloxycarbonyl,
N'-t-butyloxycarbonyl-L-cystine
ethyl, t-butyl diester (75)

To the thiolsulfinate 74 (2.31 g, 4 mmol) in benzene (40 ml) was added hexaethylphosphorus triamide (0.99 g, 4 mmol) under an atmosphere of nitrogen. N-t-Butyloxycarbonyl-L-cysteine t-butyl ester (1.11 g, 4 mmol) was added, and the mixture stirred under an atmosphere of nitrogen for 5 hr.

Solvent was removed in vacuo and the resulting oil purified on the mplc with 10% acetone in hexane as the eluting solvent system to give 1.23 g (55%) of 75 as an oil; tlc (solvent B) Rf 0.31; ^1H NMR (90 MHz, CDCl_3) δ 1.3 (t, 3 H, ethyl ester methyl), 1.5 (s, 18 H, Boc and t-butyl Me's), 3.2 (t, 4 H, cystine methylenes), 4.3 (q, 2 H, ester methylenes), 5.1 (s, 2 H, benzyloxycarbonyl methylenes), 5.4-5.8 (d, 2 H, NH), 7.4 (s, 5 H, benzyloxycarbonyl aromatics). (Found: C, 54.00; H, 7.00; N, 5.05; S, 11.57. $\text{C}_{25}\text{H}_{38}\text{O}_8\text{N}_2\text{S}_2$ requires: C, 53.76; H, 6.81; N, 5.02; S, 11.47 %).

Synthesis of lanthionine 76

To cystine 75 (0.96 g, 1.74 mmol) in benzene (25 ml) was added hexaethylphosphorus triamide (0.47 g, 1.91 mmol), at room temp. under an atmosphere of nitrogen, and the reaction mixture stirred for 5 hr.

Removal of solvent resulted in an oil which was chromatographed on the mplc with 10% acetone in hexane as the eluting solvent system to yield 0.47 g (52%) of lanthionine 76 as an oil; tlc (solvent B) Rf 0.31; ^1H NMR (60 MHz, CDCl_3) δ 1.3 (t, 3 H, ethyl ester methyl), 1.5

(s, 18 H, Boc and t-butyl Me's), 3.0 (t, 4 H, lantionine methylenes), 4.3 (q, 2 H, ethyl ester methylenes), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 5.4-5.8 (d, 2 H, NH), 7.4 (s, 5 H, benzyloxycarbonyl aromatics). (Found: C, 56.90; H, 7.31; N, 5.30; S, 6.09. $C_{25}H_{38}O_8N_2S$ requires: C, 57.03; H, 7.22; N, 5.32; S, 6.08 %).

N,N'-Di-2,2,2-trichloroethyloxy-
carbonyl-L-cystine di-t-butyl
ester 77

To L-cystine di-t-butyl ester dihydrochloride (1.28 g, 3 mmol) in H_2O (100 ml), cooled to 0° in ice-salt bath, was added $NaHCO_3$ (1.01 g, 12 mmol) and the mixture stirred for 5 min. 2,2,2-Trichloroethyl chloroformate (1.91 g, 9 mmol) was added to the cold mixture dropwise, with stirring. The reaction mixture was stirred at 0° for 2 hr followed by stirring at room temp. for 5 hr.

The reaction mixture was acidified in cold with 6 N HCl, saturated with NaCl and extracted with ethyl acetate (4 x 25 ml). The combined organic phase was washed with H_2O , sat NaCl aq and dried over Na_2SO_4 . Removal of solvent led to an oil, which was purified on mpc to yield 1.76 g (84%) of 77; tlc (solvent B) R_f 0.56, 1H NMR (90 MHz, $CDCl_3$) δ 1.5 (s, 18 H, t-butyl ester Me's), 3.2 (d, 4 H, cystine methylenes), 4.6 (m, 2H, α -H's), 4.8 (s, 4 H, trichloroethyloxycarbonyl methylenes), 6.0 (d, 2 H, NH's). (Found: C, 34.27; H, 4.38; N, 3.85; S, 9.03. $C_{20}H_{30}N_2S_2Cl_6O_8$ requires: C, 34.14; H, 4.27; N, 3.98; S, 9.10%).

Synthesis of thiol sulfinat 78

To 1.27 g (1.8 mmol) of 77 dissolved in CH_2Cl_2 (40 ml) and cooled to 0° in ice-salt bath was added m-chloroperbenzoic acid (0.44 g, 2.16

mmol) slowly in portions. The clear solution was turbid after addition of the acid. The reaction mixture was allowed to warm up to room temp. with stirring, upon which a clear solution resulted. The mixture was allowed to stand at room temp. overnight.

The reaction mixture was washed with sat NaHCO_3 aq (3 x 25 ml). The combined organic phase was washed with brine, dried over Na_2SO_4 and solvent removed in vacuo to yield 1.12 g (85%) of 78 as an oil. This oil was used without further purification for subsequent reactions; tlc (solvent B) Rf: 0.24.

Synthesis of N-benzyloxycarbonyl-L-cysteine ethyl ester

To N,N'-di-benzyloxycarbonyl-L-cystine diethyl ester (1.69 g, 3 mmol) dissolved in a 5% solution of acetic acid in diethyl ether (100 ml), and cooled to 0° in ice-salt bath, was added zinc dust (5.81 g, 180 mmol) slowly, in portions, over a period of 15 min. The mixture was stirred at 0° for 2 hr and at room temp. for one hr.

The reaction mixture was filtered and the ppt washed well with acetic acid:ether. The combined filtrate and washings were concentrated in vacuo, and the residue dissolved in 1 N HCl (50 ml). The aqueous solution was saturated with NaCl and extracted with ethyl acetate (3 x 25 ml). The organic phase was dried over Na_2SO_4 to yield 1.69 g (100%) of title compound as an oil. This oil was used without further purification for subsequent reactions; tlc (solvent A) Rf: 0.3; ^1H NMR (60 MHz, CDCl_3) δ 1.3 (t, 3 H, ethyl ester Me), 1.9 (d, 1 H, SH), 3.1 (dd, 2 H, cysteine methylenes), 4.3 (q, 2 H, ester methylenes), 4.8 (m, 1 H, α -H), 5.2 (s, 2H, benzyloxycarbonyl methylenes), 5.9 (bd, 1 H, NH), 7.4 (s, 5 H, benzyloxycarbonyl aromatic).

Synthesis of N-benzyloxycarbonyl,
N'-2,2,2-trichloroethyloxycarbonyl-
L-cystine ethyl, t-butyl diester (79)

To a solution of thiol sulfinate 78 (2.01 g, 2.8 mmol) in benzene (40 ml), through which nitrogen gas had been bubbled through for 5 min, was added hexaethylphosphorus triamide (0.72 g, 3.0 mmol) at room temp. under nitrogen, followed by N-benzyloxycarbonyl-L-cystine ethyl ester (0.84 g, 4 mmol), and the mixture stirred at room temperature for 8 hr. The solvent was removed in vacuo and the resulting oil purified on the mplc with 10% acetone in hexane as elutant to yield 0.90 g (54%) of 79 as an oil; tlc (solvent hexane-acetone, 9:1) Rf 0.2; ^1H NMR (60 MHz, CDCl_3) δ 1.3 (t, 3 H, ethyl ester Me), 1.5 (s, 9 H, t-butylester Me), 3.2 (d, 2 H, cystine methylenes), 4.3 (q, 2 H, ethyl ester methylenes), 4.6 (m, 2 H, α -H's), 4.8 (s, 2 H, trichloroethyloxycarbonyl methylenes), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 5.9 (dd, 2 H, NH's), 7.4 (s, 5 H, benzyloxycarbonyl aromatics). (Found: C, 43.25; H, 4.96; N, 4.54; S, 9.63. $\text{C}_{23}\text{H}_{31}\text{N}_2\text{O}_8\text{Cl}_3\text{S}_2$ requires: C, 43.57; H, 4.89; N, 4.42; S, 10.10%.)

Synthesis of lanthionine 80

To a solution of 79 (0.33 g, 0.54 mmol) in benzene (15 ml) was added hexaethylphosphorus triamide (0.34 g, 1.36 mmol) dropwise, with stirring, under nitrogen. The reaction mixture was stirred at room temp. overnight, and evaporated in vacuo to an oil. The oil was purified on the mplc with 10% acetone in hexane as the eluting solvent, to yield 0.18 g (58%) of 80 as an oil; tlc (solvent hexane-acetone, 9:1) Rf: 0.22; ^1H NMR (90 MHz, CDCl_3) δ 1.3 (t, 3 H, ethyl ester Me), 1.5 (s, 9 H, t-butyl ester Me), 3.0 (d, 2 H, lanthionine methylenes), 4.2 (q, 2 H, ethyl ester methylenes), 4.6 (m, 2 H, α -H's), 4.8 (s, 2 H,

trichloroethyloxycarbonyl methylenes), 5.2 (s, 2 H, benzyloxycarbonyl methylenes), 5.9 (dd, 2 H, NH's), 7.4 (s, 5 H, benzyloxycarbonyl aromatics). (Found: C, 47.16; H, 5.27; N, 4.75; S, 5.59. $C_{23}H_{31}N_2O_8Cl_3$ requires: C, 45.88; H, 5.15; N, 4.66; S, 5.32%). Two attempts to obtain satisfactory analysis for 80 resulted in % C found to be higher than the calculated value.

Synthesis of N-2,2,2-trichloro-
ethyloxycarbonyl-L-cysteine
t-butyl ester (86)

To 77 (0.37 g, 0.53 mmol) in 90% methanol aq (5 ml) was added tri-n-butyl phosphine (0.312 g, 1.05 mmol) and the reaction mixture was stirred at room temp. for 5 hr. The solvent was removed in vacuo and the residue triturated with ether (10 ml). Removal of ether led to an oil which was purified on a silica gel gravity column with 5% acetone in hexane as the elutant. Compound 86 was obtained, 0.33 g (89%), as an oil; tlc (solvent hexane-acetone, 9:1), R_f 0.3, 1H NMR (60 MHz, $CDCl_3$) δ 1.5 (s, 9 H, t-butyl ester Me's), 1.9 (d, 1 H, SH), 3.2 (dd, 2 H, cysteine methylenes), 4.8 (s, 2 H, trichloroethyloxycarbonyl methylenes).

Alternate method of synthesis
of 79

To thiolsulfinate 74 (1.03 g, 1.78 mmol) in benzene (20 ml) was added hexaethylphosphorus triamide (0.44 g, 1.78 mmol) under nitrogen. N-2,2,2-trichloroethyloxycarbonyl-L-cysteine t-butyl ester was added, and the mixture stirred under N_2 for 6 hr.

Solvent was removed in vacuo and the resulting oil purified on the mplc with 10% acetone in hexane to yield 0.70 g (65%) of 79 as an oil. This oil was identical to the one prepared by the previous method.

BIBLIOGRAPHY

- ¹H. E. Carter, C. P. Schaffner and D. Gottlieb, Arch. Biochem. Biophys. **53**, 282 (1954).
- ²M. Ueda, Y. Tanigawa, Y. Okami and H. Umezawa, J. Antibiot. Ser. A. **7**, 125(1954).
- ³T. Yoshida, K. Katgiri and S. Yokozawa, J. Antibiot. Ser. A. **14**, 330 (1961).
- ⁴J. Stop and K. Katagiri, J. Antibiot. Ser. A. **14**, 335 (1961).
- ⁵K. Katagiri, T. Yoshida and K. Sato, Antibiotics: Mechanism of Action of Antimicrobial and Antitumor Agents, (Edited by J. W. Corcoran and F. E. Hahn), Vol. 111. pp. 234-251, Springer, Heidelberg (1975).
- ⁶K. Nittka, J. Antibiot. Ser. A. **14**, 120 (1955).
- ⁷K. Katagiri, H. Endo, M. Toda and H. Nikaido, Ann. Rept. Shiongi Res. Lab. **16**, 58 (1966).
- ⁸A. Tsunoda, J. Antibiot. Ser. A. **15**, 60 (1962).
- ⁹R. Corboz, L. Ettinger, E. Gaumann, W. Keller-Schierlein, F. Kradolfev, L. Niepp, V. Prelog, P. Reusser and H. Zahner, Helv. Chim. Acta **40**, 199 (1957).
- ¹⁰K. Sato, Y. Niinomi, K. Katagiri, A. Matsukage and T. Minagawa, Biochim. Biophys. Acta **174**, 230 (1969).
- ¹¹K. Katagiri and K. Sugivra, Antimicrobial Agents Chemotherapy 162 (1961).
- ¹²S. Matsuura, J. Antibiot. Ser. A. **18**, 43 (1963).
- ¹³Reported by the National Cancer Institute in DCT Newsletter, Number 5, June 15, 1978, p. 4.

- ¹⁴R. K. Olsen, Chemistry of Amino Acids, Peptides and Proteins, (Edited by B. Weinstein) Vol. VII. (In press), Marcel Dekker Inc., New York (1983).
- ¹⁵P. K. Chakravarty and R. K. Olsen, Tetrahedron Letters **19**, 1613 (1978).
- ¹⁶J. S. Lee and M. J. Waring, Biochem. J. **173**, 115 (1978).
- ¹⁷J. Lee and M. J. Waring, Ibid. **173**, 129 (1978).
- ¹⁸M. J. Waring and L. P. G. Wakelin, Nature **252**, 653 (1974).
- ¹⁹M. A. Viswamitra, O. Kennard, W. B. T. Cruse, E. Egert, P. G. Sheldrick, M. J. Waring, L. P. G. Wakelin and R. K. Olsen, Nature **289**, 817 (1981).
- ²⁰J. R. Kalman, T. J. Blake, D. H. Williams, J. Feeney and G. C. K. Roberts, J. Chem. Soc. Perkin I, 1313 (1979).
- ²¹T. L. Ciardelli, P. K. Chakravarty and R. K. Olsen, J. Am. Chem. Soc. **100**, 7684 (1978).
- ²²M. J. Waring, Curr. Chemother. Immunother, Proc. Int. Congr. Chemother. **12th** **2**, 1370 (1981).
- ²³W. Keller-Schierlein, M. L. Mihailovic and V. Prelog, Helv. Chim. Acta **42**, 305 (1959).
- ²⁴A. Dell, D. H. Williams, H. R. Morris, G. A. Smith, J. Feeney and G. C. K. Roberts, J. Am. Chem. Soc. **97**, 2497 (1975).
- ²⁵D. G. Martin, S. A. Mizsak, C. Biles, J. L. Stewart, L. Baczynskyj and P. A. Meulman, J. Antibiot. **28**, 332 (1975).
- ²⁶H. Otsuka and J. Shoji, Tetrahedron **21**, 2931 (1965).
- ²⁷Y. Kyoguko, N. Higuchi, M. Shin, K. Inoye and H. Otsuka, Pept. Chem. **18**, 95 (1980).

- ²⁸Y. Kyoguko, N. Higuchi, M. Shin, K. Inoye and H. Otsuka, Pept. Chem. **18**, 95 (1980).
- ²⁹K. Kawano, N. Higuchi and Y. Kyoguko, Pept. Chem. **14**, 93 (1976).
- ³⁰N. Higuchi, M. Watanabe and K. Kawano, Biopolymers **20(9)**, 1959 (1981).
- ³¹M. P. Williamson and D. H. Williams, J. Chem. Soc. Chem. Commun. **4**, 165 (1981).
- ³²G. Ughetto and M. J. Waring, Mol. Pharmacol. **13**, 579 (1977).
- ³³H. T. Cheung, J. Feeney, G. C. K. Roberts, D. H. Williams, G. Ughetto and M. J. Waring, J. Am. Chem. Soc. **100**, 46 (1978).
- ³⁴T. J. Blake, J. R. Kalman and D. H. Williams, Tetrahedron Letters 2621 (1977).
- ³⁵K. Kawano, S. Higuchi and Y. Kyoguko, Proceedings of the 14th Peptide Symposium, (Edited by K. Nakajima), pp. 93-98. Peptide Research Foundation, Minoh. (1976).
- ³⁶T. L. Ciardelli, P. K. Chakravarty and R. K. Olsen, J. Am. Chem. Soc. **100**, 7684 (1978).
- ³⁷R. B. Woodward, K. Heusler, J. Bosteli, P. Nageli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbruggen, J. Am. Chem. Soc. **88**, 852 (1966).
- ³⁸D. F. Verber, J. D. Milkowski, S. L. Varga, R. G. Denkwalter and R. Hirschmann, J. Am. Chem. Soc. **94**, 5456 (1972).
- ³⁹T. L. Ciardelli and R. K. Olsen, J. Am. Chem. Soc. **99**, 2806 (1977).
- ⁴⁰B. Kamber, Helv. Chim. Acta. **54**, 927 (1971).

- ⁴¹a. F. C. McKay and N. F. Albertson, J. Am. Chem. Soc. **79**, 4686 (1957).
- b. G. W. Anderson and A. C. McGregor, Ibid. **79**, 6180 (1957).
- c. L. A. Carpino, Ibid. **82**, 2725 (1960).
- ⁴²E. Schnabel, H. Klostermeyer and H. Berndt, Justus Leibigs Ann. Chem. **749**, 90 (1971).
- ⁴³W. Chen, M. Hsu and R. K. Olsen, J. Org. Chem. **40**, 3110 (1975).
- ⁴⁴H. C. Koppel, I. L. Honigberg, R. H. Springer and C. C. Cheng, J. Org. Chem. **28**, 1119 (1963).
- ⁴⁵M. K. Dhaon, J. H. Gardner and R. K. Olsen, Tetrahedron **28**, 57 (1982).
- ⁴⁶E. J. Corey and S. Bhattacharya, Tetrahedron Letters 3919 (1977).
- ⁴⁷P. K. Chakravarty and R. K. Olsen, J. Org. Chem. **43**, 1270 (1978).
- ⁴⁸a. J. C. Sheehan, P. A. Cruikshank and G. L. Boshart, J. Org. Chem. **26**, 2525 (1961).
- b. J. Halstrom and H. Klostermeyer, Justus Leibigs Ann. Chem. **715**, 208 (1968).
- c. W. Konig and I. Geiger, Ibid. **727**, 125 (1969).
- ⁴⁹M. Shin, K. Inouye and H. Otsuka, Bull. Chim. Soc. Jpn. **51**, 1501 (1978).
- ⁵⁰J. Meinhofer, J. Am. Chem. Soc. **92**, 3771 (1970).
- ⁵¹M. Shin, K. Inouye, H. Otsuke, N. Higuchi and Y. Kyogokum, Pept. Chem. **18**, 207 (1980).
- ⁵²H. A. Staub, Justus Leubugs Ann. Chem. **609**, 75 (1957).
- ⁵³S. Akabori, S. Sakivara and M. Shin, Proceedings of the 2nd Symposium on Peptide Chemistry. pp. 39-45, Institute for Protein Research, Osaka University (1963).

- ⁵⁴R. K. Olsen, J. Heterocyclic Chem. **7**, 435 (1970).
- ⁵⁵a. R. B. Merrifield, J. Am. Chem. Soc. **85**, 2149 (1963).
b. R. B. Merrifield, Biochemistry **3**, 1385 (1964).
c. J. M. Steward and J. D. Young, Solid Phase Peptide Synthesis. pp. 151-155, W. H. Freeman and Co., San Francisco, California (1969).
- ⁵⁶M. J. Horn, D. B. Jones and S. J. Ringel, J. Biol. Chem. **138**, 141 (1941).
- ⁵⁷M. J. Horn and D. B. Jones. J. Biol. Chem. **139**, 47 (1941).
- ⁵⁸Y. Tanigawa, H. Kanamaru and S. Murahashi, Tetrahedron Letters **52**, 4655 (1975).
- ⁵⁹T. Mukaiyama, T. Masui and T. Izawa, Chem. Lett. 1177 (1976).
- ⁶⁰M. Furukawa, T. Suda and S. Hayashi, Synthesis **4**, 282 (1974).
- ⁶¹I. Degani, R. Fochi and V. Regondi, Synthesis **3**, 178 (1979).
- ⁶²T. Kubota, S. Miyashita, T. Kitazume and N. Ishikawa, Chem. Lett. 845 (1979).
- ⁶³V. du Vigneaud and G. B. Brown, J. Biol. Chem. **134**, 413 (1940).
- ⁶⁴H. I. Jacobsen, R. G. Harvey and E. V. Jensen, J. Am. Chem. Soc. **77**, 6064 (1955).
- ⁶⁵D. N. Harpp and J. G. Gleason, J. Org. Chem. **36**, 73 (1971).
- ⁶⁶I. Photaki, I. Samouilidis, S. Caranikas and L. Zervas, J. Chem. Soc. Perkin I, 2599 (1979).
- ⁶⁷M. Wilchek, C. Zioudrou and A. Patchornik, J. Org. Chem. **31**, 2865 (1966).
- ⁶⁸C. Zioudrou, M. Wilchek and A. Patchornik, Biochemistry **4**, 1811 (1965).
- ⁶⁹K. Nakajima, H. Oda and K. Okawa, Bull. Chim. Soc. Jpn. **56**, 520 (1983).

- ⁷⁰D. N. Harpp, K. Steliou and T. H. Chan, J. Am. Chem. Soc. **100**, 1222 (1978).
- ⁷¹D. A. Armitage, M. J. Clark and C. C. Tao, J. Chem. Soc. Perkin I 680 (1972).
- ⁷²S. J. Brois, J. F. Pilot and H. W. Barnum, J. Am. Chem. Soc. **92**, 7630 (1970).
- ⁷³K. C. Mattes and O. L. Chapman, J. Org. Chem. **42**, 1814 (1977).
- ⁷⁴L. Field, Synthesis 107 (1970), and references cited therein.
- ⁷⁵S. Oae, Y. K. Kim, D. Fukushima and K. Shinham, J. Chem. Soc. Perkin I 913 (1978).
- ⁷⁶S. Oae, D. Fukushima and Y. K. Kim, J. Chem. Soc. Chem. Commun. 407 (1977).
- ⁷⁷T. Endo, H. Tasai and T. Ishigami, Chem. Lett. 813 (1975).
- ⁷⁸S. Oae, Y. H. Kim, D. Fukushima and T. Takata, Chem. Lett. 893 (1977).
- ⁷⁹B. Zwanenburg and P. Kielbasinski, Tetrahedron **35**, 169 (1979).
- ⁸⁰L. Field and R. Ravichandran, J. Org. Chem. **44**, 2624 (1979).
- ⁸¹B. Kamber, Helv. Chim. Acta **56**, 1370 (1973).
- ⁸²B. Kamber, Helv. Chim. Acta **54**, 927 (1971).
- ⁸³D. S. Kemp, S. Leung and D. J. Kerkman, Tetrahedron Letters **22**, 181 (1981).
- ⁸⁴R. G. Hiskey and B. F. Ward, Jr., J. Org. Chem. **35**, 1118 (1970).
- ⁸⁵R. G. Hiskey, N. Muthukumraswamy and R. H. Vunnam, J. Org. Chem. **40**, 950 (1975).
- ⁸⁶a. A. Schoberl, H. Tausent and H. Grafje, Angew. Chem. **68**, 213 (1956).

- b. L. D. Small, J. H. Bartey and C. J. Cavallito, J. Am. Chem. Soc. **69**, 1710 (1947).
- ⁸⁷L. Moroder, A. hallet, E. Wiinsch, A. keller and G. Wersin, Hoppe-Seyler's Z. Physiol. Chem. **357**, 1651 (1976).
- ⁸⁸a. K. D. J. Kopple, Pharmaceut. Sci. **61**, 1345 (1972).
b. E. Schroeder, L. Lubke, The Peptides, Vol. I and II. Academic Press, New York (1965, 1966).
- ⁸⁹D. Gen Ishai and A. Berger, J. Org. Chem. **17**, 1564 (1952).
- ⁹⁰I. Zervas and D. M. Theodoropoulus, J. Am. Chem. Soc. **78**, 1359 (1956).
- ⁹¹J. Buter and R. M. Kellogg, J. Org. Chem. **46**, 4481 (1981).
- ⁹²S. Hanessian and J. M Vatele, Tetrahedron Letters **22**, 3549 (1981).
- ⁹³H. A. Staab, Angew. Chem. **1**, 5 (1962).
- ⁹⁴E. Wunsch and G. Wendlberger, Chem. Ber. **97**, 2504 (1964).
- ⁹⁵C. C. Wang, R. Dimarchi and F. R. N. Gurd, Semisynthetic Peptides and Proteins (Edited by R. E. Offord and C. Dibelo), pp. 59-72. Academic Press, New York (1978).
- ⁹⁶L. Field and Y. J. Khim, J. Org. Chem. **37**, 2710 (1972).
- ⁹⁷E. Block and J. O'Connor, J. Am. Chem. Soc. **96**, 3921 (1974).
- ⁹⁸B. Boduszek and J. L. Kice, J. Org. Chem. **47**, ³¹⁹⁹3200 (1982).
- ⁹⁹S. Wolfe, P. M. Kazamaier and H. Auski, Can. J. Chem. **57**, 2412 (1979).
- ¹⁰⁰J. G. Wilson and L. A. Cohen, J. Am. Chem. Soc. **85**, 560 (1963).
- ¹⁰¹E. Block, J. Chem. Educ. **48**, 814 (1971).
- ¹⁰²L. Field, Synthesis **4**, 101 (1972).
- ¹⁰³E. Block and J. O'Connor, J. Am. Chem. Soc. **96**, 3929 (1974).

- ¹⁰⁴J. Goodacare, R. J. Ponsford and I. Sterling, Tetrahedron Letters 3609 (1975).
- ¹⁰⁵H. Yajima, H. Watanabe and M. Okamoto, Chem. Pharm. Bull **19**, 2183 (1971).
- ¹⁰⁶B. W. Erickson and S. A. Khan, J. Am.Chem. Soc. **103**, 7734 (1981).
- ¹⁰⁷H. C. J. Ottenheijm, R. M. J. Liskamp, S. P. J. M. van Nispen, H. A. Boots and M. W. Tijhuis, J. Org. Chem. **46**, 3273 (1981).
- ¹⁰⁸E. J. Corey and S. Bhattacharyya, Tetrahedron Letters 3919 (1977).
- ¹⁰⁹B. Marinier and M. Berube, Can. J. Chem. **50**, 1638 (1972).

APPENDIX

Abbreviations

The abbreviations used in this dissertation are as follow:

Acm:	Acetamidomethyl
Bam:	Benzamidomethyl
Boc:	t-Butyloxycarbonyl
Bpa:	p-Bromophenacyl
DCC:	N,N'-dicyclohexylcarbodiimide
EDC:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBT:	1-Hydroxybenzotriazole
Tce:	2,2,2-Trichloroethyl
Toc:	2,2,2-Trichloroethyloxycarbonyl
Qxc:	Quinoxaline-2-carbonyl
Z:	Benzyloxycarbonyl

VITA

Ganesh Devdas Kini

Dissertation: Studies in Peptide Synthesis towards the
Lanthionine Analog of Des-N-Tetramethyl
Triostin A

Major Field: Organic Chemistry

Biographical Information:

Date of Birth: January 25, 1955

Place of Birth: Bombay, India

Academic Record:

High School: Graduated from Little Angels High School,
Bombay, India (1971).

Bachelor of Science (Hons.): S.I.E.S. College of Arts and
Science, affiliated to the University of
Bombay, India (1975). Major: Chemistry;
Minor: Physics

Master of Science: University Department of Chemistry,
University of Bombay, India (1977).
Analytical Chemistry

Doctor of Philosophy: Completed requirements for Doctor of
Philosophy degree at Utah State University,
1983.